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- (C) UNITS:
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- :A NAME KEY:
- F LOCATION:
- C: IDENTIFICATION METHOD:
- (D) OTHER INFORMATION:
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 ${\tt CAATCAGCAGACAGCAGTGGTACTTATGAGGTA33}$

CLAIMS:

What is claimed is:



- 1. A numer monoclonal antibody which specifically binds to a surface antigen of a stomach cancer cell MKN 45, said antibody belonging to IgG class, and said antibody having a variable region of the heavy chain which is the amino acid sequence shown in SEQ ID No. 5 and a variable region of the light chain which is the amino acid sequence shown in SEQ ID No. 6.
- 2. A F(ak').sub.2 fragment of the human monoclonal antibody of claim 1.
- 3. A Fab' fragment of the human monoclonal antibody of claim 1.

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L1: Entry 3 of 3

File: USPT

Jun 16, 1998

US-PAT-NO: 5767246

DOCUMENT-IDENTIFIER: US 5767246 A

TITLE: Human monoclonal antibody specifically binding to surface antigen of cancer

cell membrane

DATE-ISSUED: June 16, 1998

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APPL-NO: 08/ 360125 [PALM] DATE FILED: December 20, 1994

PARENT-CASE:

This application is a continuation of now abandoned application Ser. No. 07/905,534, filed Jun. 29, 1992.

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PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search ALL Search Selected

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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FOREIGN-PAT-NO	REIGN-PAT-NO PUBN-DATE		US-CL
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ART-UNIT: 186

PRIMARY-EXAMINER: Schwadron; Ronald B.

ABSTRACT:

A human monoclonal antibody specifically binding to a surface antigen of cancer cell membrane, an isolated DNA encoding the antibody, and a hybridoma producing the antibody. An anti-cancer formulation comprising the monoclonal antibody bonded to the surface of a liposome enclosing an anti-cancer agent or toxin is also provided.

3 Claims, 5 Drawing figures Exemplary Claim Number: 1 Number of Drawing Sheets: 4

BRIEF SUMMARY:

- The present invention relates to a novel human monoclonal antibody useful for diagnosis and therapy of cancer, an isolated DNA encoding the monoclonal antibody, and a hybridoma producing the antibody. The present invention also relates to an anti-cancer formulation comprising the antibody bonded to a liposome which contains an anti-cancer agent.
- There has been no anti-cancer formulation thus far, which is sufficiently effective for the treatment of solid cancer. On the other hand, there has long

existed an idea called "targeting" in which a therapeutical agent is concentrated at a tissue or an organ to be treated in order to maximize the therapeutical effect of the agent. Accordingly, it has been expected that focusing an anti-cancer agent at a cancer tissue by means of "targeting" may allow a therapy of the solid cancer. A number of trials to concentrate an anti-cancer agent or a toxin at a cancer tissue were made since a method for production of mouse monoclonal antibodies in large quantities has been established by Milstein and Rohler (Nature, 1975), and some of them were successful.

- Inus far, binding of an antibody to a therapeutic agent has been adcomplished by directly binding an antibody to a chemically-modified therapeutic agent, or indirectly binding them via a water-voluble polymer such as dextran. These methods, nowever, have drawbacks in that the amount of a therapeutic agent capable of binding to one antibody milecule is very limited, and in that chemical modification of a therapeutic agent often causes lowering of the therapeutical activity. As one of the countermeasures to overcome the drawbacks, there was proposed a new delivery system which consists of an antibody bonded to the surface of a liposome in which a therapeutic agent is encapsuled, and many favorable results were reported (Nonno et al, Dander Research 47 4471, 1987; Hamnimoto et al, Dapanese Patent Publication (unexamined) No. 1340/2/1968).
- However, mouse monoclonal antibodies have a limited clinical use and continued administration thereof is impossible from a practical point of view due to side effects such as anaphylaxis baused by immune response (See A. Lo Bugli et al, Broc. Matl. Acad. Sci. U.S.A., 86 4200, 1989). Accordingly, human monoclonal antibodies rather than mouse monoclonal antibodies are preferable for the purpose of clinical use. However, preparation of human monoclonal antibodies which adequately react with cancer cells has long been considered very difficult because of the reasons that it is very difficult to conduct passive immunity for the purpose of obtaining number B cells which produce a desired antibody, and that any efficient methodology which allows infinite reproduction of antibody-producing cells has not been established yet.
- In such a situation as mentioned above, the inventors of the present invention have made extensive study for the purpose of obtaining a human monoclonal antibody which permits "targeting thorapy" on rander tissue or organ with the help of anti-cancer agents or toxins, and they have succeeded in preparing a hybridoma capable of producing a novel human monoclonal antibody, the antigen to which exists on the surface of cell membrane of cancer cells. They also have succeeded in preparing a therapoutical formulation useful for "targeting therapy" of cancer, by binding the minoclonal antibody of the invention to a liposome in which an anti-cancer agent is encapsuled. The present invention is based on these findings.
- Thus, the present invention provides a human moniclinal antibody specific to an antiden existing on the surface of a cancer cell membrane, said moniclonal antibody being produced by a fused cell between a lymphocyte derived from cancer patient and a mouse myeloma cell. The invention further provides an isolated gene encoding the antibody, a hybridoma producing the antibody, and an anti-cancer formulation containing the antibody.
- The number monoclonal antibodies of the present invention contain, in the variable region of the heavy chain, the amino acid sequences shown, for instance, in Sequence Listing Nos. 1%, 14, and 18. More specifically, the monoclonal antibodies of the invention include, among others, those in which the variable region of the heavy chain comprises the amino acid sequences shown in Sequence Listing Nos. 16, 1, and 18, and the variable region of the light chain comprises the amino acid sequences shown in Sequence Listing Nos. 19, 20, and 21, and those in which the variable region of the heavy chain comprises the amino acid sequences given in Sequence Listing Nos. 22, 23, and 24, and the variable region of the light chain comprises the amino acid sequences given in Sequence Listing Nos. 25, 26, and 27.

The monoclonal antibodies of the invention include any variants of the above-mentioned specific antibodies, which are obtainable by making insertion, deletion, substitution and/or addition of one or more amino acid residues to the amino acid sequences of the above-identified antibodies with the limitations that such modification must not adversely affect the reactivity of the antibodies against the antigens. The present invention will be more detailed below.

DRAWING DESCRIPTION:

In the accompanying drawings;

FIG. 1 streamatically shows the bonstruction of vector pECRD.

FIG. 2 some matically shows the construction of vector pHCR(.DELFA.E) H.

FIG. 3 shows reactivity of antibody 1-3-1 to colon cancer cell line C-1.

FIG. 4 shows reactivity of antiopdy 1-3-1 to dastrib cander cell line MEN45.

FIG. 5 shows anti-cancer effects of admissive in-containing and PEG-modified liposome bonder to untibody GAH or the cancer transplanted to nude mouse.

DETAILED DESCRIPTION:

- I'me hybridoms producing a numer monoclonal antibody of the invention is prepared according to the method described by A. Imam (Cancer Research 45 263, 1988). Thus, lymphocytes which have been isolated from extracted lymph node associated with cancer are fused with mouse myeloma cells in the presence of polyethylene glycol. Hybridomas thus obtained are screened by means of enzyme immuneassay using various cancer cell line fixed with paraformaldehyde, and hybridomas capable of producing antibodies are obtained and cultured. From supernatant of the resulting culture, monoclonal antibodies are isolated and purified according to a conventional method such as displosed by B. C. Duhamel (J. Irmunol, Methods 31 211, 1979).
- The purified monoclonal antibody is labelled with a fluorescent substance and examined about its reactivity with living cancer cells and normal cells such as erythrecytes and leucocytes using Flow Cytometry. Hybridoms projucing an antibody which reacts with the living cells but not with normal cells are selected. Alternatively, the reactivity of antibodies to cancer cells isolated from tancer tissue of a patient is compared with the reactivity to normal cells derived from non-cancer segment of the same organ, and a hybridoma producing an antibody which reacts with the cancer cell and does not react, or reacts as moderately as an antibody derived from normal volunteer, with normal cells, is selected.
- 3 A base sequence of a DNA encoding a human monorional antibody produced by the hybritoma selected above can be determined in the following manner.
- In adverdance with Casara et al method (DNA 2 129, 1983), manAs are separated from the antibody-producing hybridoms cells, using quantities thiodyanate-lithium chloride, and cDNA library is prepared by the use of oligo (dF) primer. The cDNAs thus obtained are then subjected to (dG) tailing. Consensus sequence between poly C capable of hybridizing with the dG tail obtained above and an already available numan gene encoding heavy or light chain of numan antibodies is used as a probe for amplification of the antibody-encoding cDNA by means of PCS. The terminal of the amplified DNA is made plunt. The DNA separated from an electrophoresis gel is inserted to a cloning vector such as pUC119, and the base sequence of the DNA is determined

- by Sanger et al didecky method (Proc. Natl. Acad. Sci. U.S.A. 74 5463, 1997).
- Preferable antikodies of the present invention are those in which the variable region of the heavy chain comprises the amine acid sequences shown in Sequence Listing Nos. 13, 14, and 15. Specific examples of preferred antibodies are, among others, those in which the variable region of the heavy chain comprises the amine acid sequences shown in Sequence Listing Nos. 16, 17, and 18, and the variable region of the light chain comprises the amine acid sequences shown in Sequence Listing Nos. 19, 70, and 71, and those in which the variable region of the neavy chain comprises the amine acid sequences shown in Sequence Listing Nos. 22, 13, and 24, and the variable region of the light chain comprises the amine acid sequences shown in Sequence Listing Nos. 25, 16, and 27.
- The above-noted amino acid sequences in Sequence Listing Nos. 13, 14, and 18; lt, 17, and 18; and 28, 28, and 24 are called "hyper variable region" in variable region of the neavy chain. Likewise, the amino acid sequences in Sequence Listing Nos. 19, 10, and 1; 25, 16, and 27 are called "hyper variable region" in variable region of the light chain. These regions are responsible for the specificity of the antibody and determinative to binding afformaty netween the antibody and the antipodies of the invention can have various amino acid sequences derived from different antibodies so far as it comparises the above-mentioned hyper variable regions.
- 7 The most preferred monoclonal antibodies of the invention are those in which the variable regions of the heavy and light chains are represented by the amino acid sequences of Sequence Listing Nos. 1 and ε respectively, and also II and II respectively. The DMA sequences enoughnous constant regions of the neavy and light chains are the same as those disclosed in Nucleic Acids Research 14 1779, 1886, The Journal of Boolegical Chemistry 157, 1816, 1988 and Cell 23, 197, 1880, respectively.
- The minustanal and ibidy of the invention may be prepared by culturing the hybridoma producing the antibody of the invention in eRDF or REMILEAN medium containing fetal bovine serum. Alternatively, it may also be prepared by connecting the DNAs having the base sequences in Sequence Listing No. 3, 4, 3 and No. 15, which enocde variable regions of heavy and light chains respectively, with known DMAs encoding the constant regrots as mentioned above to obtain a pair + f genes encoding the monorlonal antibody of the invention, inserting the genes into one of various known expression vectors, transforming an appropriate most cell such as IBO cell with the empression vectors, and culturing the resultant transformant. As empression vectors to be used in animal cells, there may conveniently used a combanation of PMCE (.DEDTA.E) (E and pKCED which may be constructed in the manner as shown in FIGS. 1 and 2 starting from pPCBH2 disclosed by Mishina (Nature 307 665, 1884). In more detail, a gone encoding the heavy chain, to which a Hindlil restriction site has been added, is inserted into plasmid pRCE (.DELTA.E.H. at the Hind!!! site, and a selective marker such as DHSE dene is inserted into the plasmid at Sall gite. On the other hand, a dene encoding the light chain, to both ends of which HotBI restrictain site has been added, is inserted into plasmid pRCBO at EcoBI site, and then the DHFR gene is also inserted into the plasmid at SalI site. Both of the plasmids obtained above are incorporated into a host cell such as OHO onfr.sup. - (Urlaub G. v Chasin L. A., Free. Natl. Acad. Sci. U.S.A., 7 4.16, 1 %%) by means if calcium prosphate method. The resultant transformant is cultured in .alpha.MEM medium containing no nucleatide, and grown cells are subjected to further selection for antibody-producing clones. The ortibody of the invention can be obtained and purified by culturing the selected clone, adsorbing the resulting supernatant to a column filled with Protein A supported by rerulatine or adarose, and eluting the antibody from the column.
- A liposome used for the preparation of the anti-cancer formulation of the invention is composed of two lipid layers. The lipid layer may be of monolayer or multiple layers. Constituents of the liposome are phophatidylcholine, cholesterol, phosphatidylethanolamine, etc. Phosphatidic acid, which provides

the liposome with electric charge, may also be added. The amounts of these constituents used for the production of the liposome are, for instance, 0.3-1 mol, preferably 0.4-0.6 mol of cholesterol, 0.31-0.2 mol, preferably 0.03-0.1 mol of phosphatidy.ethantlamine, 0.0-1.4 mol, preferably 0-1.15 mol of phosphatidic acid per 1 mol of phosphatidylandline.

- The liposome used in the present invention may be prepared by conventional methods. For example, a mixture of the above-mentioned lipids, from which the solvents have been removed, is emploified by the use of a homogenizer, lyophilized, and melted to obtain multilamera liposome. Adjustment of particle size of the resultant liposomes may be conducted by altrasonication, high-speed homogenization, or pressure filtration through a membrane having uniform pore size (Hope M. 1. et al., Biochimica et Biophysica Acta 312 57, 1955). Preferable particle size of the laposomes are between 30 nm and 200 nm.
- Anti-cancer agents encapsuled in the liptsome includes parcinostatic agents such as admismycin, dadnomycin, mitomycin, displatin, vincriptine, epirubicin, methotremate, 1 Fu, and aplacinomycin, toxins such as rigin A and diphtheria toxin, and antisense RNA. Encapsulation of anti-cancer agent into liposome is accomplished by hydration of the lipids with an aqueous solution of the anti-cancer agent. Admismycin, dadhomycin, and epirubicin may be encapsulated into a liposome by means of remote loading method taking advintage of pagratient (Lawrence D. M. et al., Jancer Research 49 8928, 1969).
- Binding of a monoclonal antibody to the surface of the liposome mentioned above may be appemplished by the formation of press-linkage between prosphatidylethanolamine and the antibody using glutarialdenyde. However, preferred method is that a thiolated antibody is allowed to react with a liposome comprising a lipid into which a maleimide group has been incorporated. Semaining maleimide groups on the surface of the liposome may be further reacted with a compound containing thiolated polyalkylenegly of molety, thoseby the surface of the lipisome is modified.
- Thiolation of an antichous may be conducted by the use of N-succinomicyl-3-(3-pyricyldithio)propionate (SPIP), which is usually used for thiolation of protein, iminothiclane, or mercaptualkylimidate. Alternatively, a mithicl group intrinsic to an antibody may be reduced to form a thiol group. The latter is preferred from the view point of keeping antibody's function. Another method to provide an antibody with a thicl group is that an antibody is treated with an engyme such as pepsin to form F(ab)'.sub.2, which is then reduced with distinctive tol (DTT) to form Fab', which gives one to three thick groups.
- The binding of the thiclated antibody to the maleimide droup-containing liposome may be accomplished by reacting them in a neutral buffer solution at $\gamma H = 0.3-7.6$ for 2-16 hours.
- 15 The anti-parker formulation of the present invention may be prepared by means of conventional methods such as dehydration method (Japanese Patent Publication No. 80234671998) and lyophilization method (Japanese Patent Publication No. 803171989).
- The anti-cancer formulation of the invention may be administered intravascularly, peritoneally, or locally. Dosage of the formulation varies depending on the nature of particular anti-cancer agent encapsulated into the liposome. When the agent is admiamyoin, the dosage is the one corresponding to admiamyoin 10 mg or less/kg body weight, preferably 10 mg or less/kg, more preferably 5 mg or less/kg.
- 17 The following detailed examples are presented by way of illustration of certain specific embodiments of the present invention.
- 18 EXAMPLE 1

- 19 Establishment of Hybridoma Producing Human Monoplonal Antibody GAH
- 20 Hybridema producing human monoclonal antibody GAH was established by cell fusion between lymphocytes derived from a lymphocytes derived from a lymphocytes derived traste of a patient and mouse myeloma cells.
- 21 (1) Ereparation of Lymphodytes
- Cancer-assiciated lymph node extracted from a patient suffering from colon cancer was dit up into fine pieces with scissors and scalpel, and colls were dispersed using a stainless net in Culture Medium A (eBDF (Kyokuto Deiyaku Kogyo)+80 .ml.g/ml.gontamicin sulfate). The resultant cell suspension was contrifuged at 1000 rpm for 10 minutes and the supernatant was disputed. The residue was paspended in from Culture Medium A, and the suspension was contributed adain to obtain 2.6.times.10.sup.7 cells.
- 23 (...) Cell Bus.on
- 1:4 The lymphocyte cells obtained above were subjected to cell fusion with mouse rivelima cells (1.times.10.scp.7) in the presence of bolyethylonealy sol (Boenringer-Mannheim) according to a conventional method. The fise: della were suspended into Bultura Medium Aladded with 11 .md.M hypoxanthina, 5.34 .md.M. aminopterin, 1.0 .ma.M trymidine, and 100 fetal calf serum (PCS), said medium being referred to as HAT addition medium hereinafter, at that the penalty of the lymphocytes may be 5.4. times. 10. \sup 5 / \min 1. The suspension was plated on 96 well plates at 100 .mm.l/well and multired at 3%.degree. C. in a 70.sub.. incubator. Half of the culture medium was substituted with HAT add: lon medium from time to time and the pultivation was continued until hybridoma's polonies appeared. The hybridima's colonies were observed in all of the wells. The supernatant of the culture in each well was tested on the reactivity to several established ranger dell lines sion as polin danger dell line C-1 (Jato et ab. Idakundayumi (Brigness of Medicine) 96 176, 1976, obtained from Men Eki Scibutsu Menkyusho (Institute of Immunized Organisms)), and stomach dander cell ine MKN45 (Maito et al, Gan to Kadaku Sycho (Cander and Chemotherapy) 5 89, 1978, obtained from above-noted Institute) according to the method described in Experiment 1. Positive wells were 7.3% (35 wells) against C-1 and 4.6% (72 wells) against MEN48, and C wells showed positive reaction to both strains. Clining of hybridimas was bondanted using the wells which showed positive twaction to poth links. The closing was bondpoted three times by means of limiting all tiln method, and nybritima flone GAB was established.
- 25 EMAMEDE 2
- 26 Purification and Labeling of Moncolonal Antibody GAM
- 27 (1) Culture of Hybridoma GAB and Purification of Monoclonal Antibody GAB
- Setal calf serim was passed through a Brotein A-agarose (Replicen), thereby substances alsorbed to the column was removed from the serum. For culture of hybridoma GAH, eRDF culture medium (Hypkuto Seiyaku) to which Be of the above serum had been added was used. The culture of hybridoma GAH was then charged into a Brotein A-agarose column, and advorbed antibody was then eluted out to obtain purified antibody. The use of the above-noted serum allowed to obtain pure antibody GAH free from other anticodies of serum origin and substances alsorbed to Protein A-agarose. The antibody GAH was confirmed to be a pure IgG by sodium dolecyl sulfate-polyacrylamide gel electrophoresis.
- 29 (2) Fluorespent Labeling of Antipody GAH
- 30 The purified antibody GAH was labeled by fluorescein isotniogyanath (FITC) according to the method of Coons A. H. Thus, the antibody was dialyzed against a carbonate buffer solution (pH 9.5) and reacted with FITC solution. The

labeled antibody was separated from free FITC by gel filtration. Absorbance of fractions containing labeled antibody was measured at CO.sub.200 nm and OD.sub.405 nm and labeling degree was determined. The kinding mola: ratio of the antibody and FITC (F/E ratio) was 0.93.

- 31 EMPERIMENT 1
- 32 Study on Reactivity of Human Monop. Small Antibody against Cancer Ce.l Lines
- 33 .1) Cander Cett Lines and Preservation Thereif
- 34 Octon pancer cell line C-1 and stomach cancer cell line MEN45 were used as numer cancer cell lines. The cells were preserved and grown at 5° degree. C. under 5: CO.sob.2 conditions using Culture Medium B (eED8 medium containing 10 e PCS).
- 35 (2) Study on Reactivity to Cander Ceil Lines
- 36). Determination of reactivity against solid cancer cell lines
- 37 Canber dells were cultured until monolayer in a 96 well plate for % or 4 days. After removal of culture supernatant, the phate was washed twide by 10 mM prosphate puffer (pH 7.4) and 1.15M MaCl silution (PBS), and 2 paraformaldehyde fixation was conducted at room temperature for 20 minutes. After washing 5 times with PBC, PBC solution containing 5% BCA (begins serum albumin) was added to wells (200.mu. well), and the plate was kept 57.degree. C. for 2 hours to complete blocking. The plate was washed 5 times with PBS, and 30 .mu.l of culture supermatant of hybridoma was added thereto. After two hour reaction at 37.degree. C., the plate was washed 5 times with EBS and 50 .mu.l of alkaliprosphatase conjugated doat antibody to human antibody (1900 dilution, Tape!) was added. Polliwing one hear reaction at 37.degree. C., the plate was rashed times with PBS and aided with 1.13M parbonate buffer--1 nM MgCl (pH 4.5) containing 25 mM p-nitrophenyl phosphate at ratio of 30 .mu.l well and allowed to readt at room temperature for one nour to overnight. Absorbance at 405 nm was measured with midri-plate photometer (Colina). Reactivity was determined according to the mothod described in Example 1 (2). Cloning from the wells in which positive reaction against cultured cancer cell wines C-1 and MENAS has been observed gave hybridoma GAH. Purified antibody from culture Purernatant o: GAH showed the same reactivity.
- 38 m. Reactivity to living dance: cells
- 39 Cancer cells were cultured in a flask or Estri dish and culture supernatant was discarded. To the residue was edded a PBS solution containing $0.02\pm {
 m EDTA}_{
 m c}$ and the mixture was left to stand at room temperature for of minutes allowing the tills to float. The cells were washed with Culture Medium B by centrifugation and suspended in healthy numar serum containing the fluorescent-labeled entibody SAH (final concentration: fr.mu.g/ml) obtained in Example 2 (2) so that cell density of about 1.times.11.sup.6 $\times 300$.mu.l may be obtained, and the Juspension was allowed to reast at 0.degree. 3. for 60 minutes. The suspension was centrofuged at 3000 rpm for 2 minutes and the supernatant was disparsed. The remaining cells were suspended in 1 ml of FBS, washed by contribugation, and resuspended in 300 .mu.l of FBS containing 10 .mu.g/ml of propidium indude (PI). The suspension was subjected to the chservation by flow dytometer (FCM), FACS440 (Beston Dickinson), in order to determine the magnitude of fluorescence 'FITC and PI) bonded to particular cell. Dead cells having PI fluorescence spuld be removed because the lead bells took in PI in the nubleic abids and emitted PI fluorespende. Markers having five standard amounts of fluorespende quantitative kit: Ortho Diagnostic Systems) were subjected to FCM under the same conditioned as above. Based on the markers, average binding amount of FITC per cell was calculated. On the basis of the average binding amount and F/F ratio of labeled antibody, an average number of antibodies bonded to one living cell was determined. The results are shown in Table 1.

TABLE 1

	Antib	 ody	_
Cancer Cel	l Strain GAH	Control IgG	
MK1145	3.5.	.times. 10.sup.4	- 10 sum 4
Q=1.	0.6	.times. 10.sup.4	

- 40 When compared with IgG derived from healthy human serum, which was labeled by fluorescence in the same manner as GAH and used as a control, about 6-23 times larger amount of antibody GAH has bonded to stomach and colon cancer cells.
- 41 EXPERIMENT 2
- 42 Reactivity of Human Monoclonal Antibody GAH to Blood Cells
- 43 Erythropytes were separated from peripheral blood taken from 7 healthy voluntuers and 3 patients suffering from cancer according to Kinoshita's method (Separation of Erythropytes; New Edition of Nippon Ketsuckigaku Zensho 13 800, 1979).
- 144 Leukocytes were obtained in the following manner: Peripheral blood was drawn from healthy volunteers with addition of neparin. 2 ml of 6% dextran-physiological saline was added and mixed to 10 ml of the blood. The nixture was left to stand at doom temperature for 30 minutes to give a plasma layer, which was then separated and centrifuged at 1500 mpm for 5 minutes to obtain leukocytes.
- 45 Reactivities of the monoclonal antibody of the invention to these blood cells were determined by means of FCM in the same manner as in the living cancer cells except that PI was not added. In this connection, the leukocytes were divided into lymphocyte major leukocyte cell), granulocyte, monocyte, and platelet, based on front and side light scattering in FCM (Bio/Technology 3 337, 1988), and reactivities to respective cells were separately determined. The test results were shown in Table 3.

TABLE I

	Antibody	
Cells	GAH	Control 196
Leukonyte		
lymphobyte	negatîre	negative
ghahulohyte	0.49 . im	ies. 10.sup.4 *
		0.48 .times. 10.sup.4 *
monocyte	0.41 . im	es. 10.sup.4 *
		0.43 .times. 10.sup.4 *
platelet	negative	negative
Erythrosyte	negative	n⊣gative
*Average nu	mber of ant	ibodies bonded per cell-

46 Antibody GAH showed no reaction to erythrocyte and lymphocyte, while the

reactivity to granulocyte and monocyte was the same level as the reactivity to control $\log C$ likewise in Experiment 1.

- 47 EXPERIMENT 3
- 48 Reactivity of Human Monoclonal Antibody GAH to Cells Derived from Fresh Cancer Tissue and Non-Cancer Tissue
- In order to study a kinding specificity of antibody GAB to cancer cells, normal cells were simultaneously isolated from fresh tissue belonging to the same crgan of the same patient from which cancer cells were obtained, and reactimities of antibody GAB to respective cells were determined. Isolation of cells from the tissue was conducted according to Tokita's method (Ganno Rinsho (Cancer in Clinic) 12 1803, 1886).
- Thus, the tissue extracted was placed on Teflon sheet spreaded on a rubber plate, but with a razor into fine pieces, and transferred onto a 1 mm stainless meshes. The meshes was shaken in a Petr: dish full of a guiture medium to chtain the nedium containing small cell aggregates which passed through the meshes. The medium was centrifuged at 1000 rpm, and floating fats and suspending heorotic dobris were discarded. This centrifugation was repeated several times. The cell aggregates were subjected to pumping by means of a springe with Cateran needle of 23 gauge to disperse the cells. The reactivity to the cells thus obtained was determined by FCM in the same manner as in the living cander cells. The test results are shown in Table 3.

TABLE 3

Stomach Non-dander Cander Non-dander Cancer Antibody Cells Calls Cella Ceils 1.1 .times. 15.sup.4 0.03 .times. 10.sup.4 180 .times. 10.sup.4 4.6 .times. 10.sup.4 Control 0.15 .times. 10.sup.4 0.04 .times. 10.sup.4 3.5 .times. 10.sup.4 7.9 .times. 10.sup.4 136 Average number of antibodies bouded per cell

- The average number of GAH antibodies bonded to cancer cells is remarkably higher than that in the non-cancer cells. In addition, the number of antibodies bended to cancer cells was 51 times greater than that in the control IgG in stomach cancer, and 7 times greater in solon cancer. These results indicate that antibody GAH recognizes an antigen dominantly expressed on the surface of cell membrane of cancer cells.
- 52 EXAMPLE 3
- 53 (1) Determination of Subclars of Light Chain of Monsolonal Antibody GAH
- Antibody GAH obtained in Example 2 (1) was subjected to SDS-PAGE in the reduced form. Heavy chain and light chain separately electrophorated were blotted on a transmembrane (Polyvinylidene-dilluoride, Millipore). The membrane was blocked with 5% BSA solution and allowed to react with a goat antibody to human .kappa. or .lambda. chain, which was combined with peroxidase (Capel). After washing, a

6.05% (w/v) 4-chlorenaphthol solution containing 0.015% H.sub.2 O.sub.2 was allowed to react thereto as a substrate. The light chain of antibody GAd reacted with anti-human .kappa. chain antibody, which was detected through the appearance of oclored band. This revealed that the light thair was .kappa. chain.

- 55 2) Preparation of Gene Encoding Moncolonal Antibody GAH
- 56 a. Preparation of cINA endoding antibody GAH by means of polymerase chain reaction (PCE)
- According to the method detailed below, poly(A)-containing RNAs were prepared from antibody GAH-producing hybridens obtained in Example 1 2' using quantities this dwarate-lithium chlorade method (DNA 0.324, 1987).
- 58 The hyperidoma cells (1.times.10.sup.7) were solubilized in a solution 7.5 ml) comprising 3M quanturne thirdyana's, 1° mM EDCA, 50 mM Tris-HCl, pH 7.0, and 88 v(v) , beta.-mercaptoethanol. To the mixture was further accord and mixed design (n1) rude to the final consentration of 1 v(1) ml. The solution (3.0 ml) was gently overlayed on a 5.7M resign coloride solution (3.5 ml) in a pentrifuge tupe, and centrifiges at 30,000 rpm for Lo.5 hours using MitAchi RPS40T Retary, which gave ENAs as a precipitate. The precipitate was dissolved in a solution .mu.l) domprising 1.1% addium laury, sultate, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.5, followed by prenti-thioroform extraction and ethanol precipitation. The resultant BMAs (about 64 .mu.d) was dissolved in a solution 1.41 .mu.l) comprising 10 nM Tris-HCl, pH \pm .7, and 1 mM ESTA. A 21 .mi.l aliquot of the solution provided about 2.64 .mi.: of mENA containing pSly(A) by means of mRNA PURIFICATION RIT (Pharmacha). The poly(A)-containing mENA (1.1 .mu.g) was dissolved in water (1) .mu.l). To the solution were added bligo d(T)=12-18rrimer (1.5 .mu.q) (Pharmacia:, 1 mM 4 dMTB -3 .mu.l) (Takara Shuzo), reverse ransprintase (40 U) (Life Science), Educe inhibitor (31 U) Takara Shuzo), ..t.mes.reverse transcriptase differ (0 .mu.l) comprising 280 rM Tris-HCl, pH e.3, 41 mM magnesium onloride, and 350 mM priassium chloride, and additionally water to make's total volume of Pilmull. The mixture was allowed to react at 41. degree. C. for one nour, followed by othered precipitation to obtain cDNA.
- The cDNA thus obtained was dissolved in water (15.5 .mu.1). To the solution were added a S.times.terminal decaynablectide transferase buffer (4.8 .mu.1) 25 mM Tris-HCl, pH 7.8, 51 mM magnesium coloridet, terminal decaynuclectide transferase (12 U) (Pharmacia , and 10 mM cQTP (8.4 .mu.1) (Takana Shubo) to make a total volume of 24 .mu.1, and the mixture was allowed to react at \$7.degree. 3. for 1.1 hours to act poly 4 G at 3' terminal of CDNA. After completion of the reaction, the ensymes word inactivated by nesting at 70.degree. 3. for 10 minutes.
- PCR was dinducted based on the SDNA thus obtained as a template using Perkin 60 Elmer Cetus DNA THERMAL Cycler following the manual provided by the manufacturer. Thus, is the above reaction mixture (. .m..1) were added, as a primer for amplifying cONA encoding variable region of the neavy chain, poly C 13 nucleations) which hybridizes d3 tail added to 3' terminal of the \$DNA (40 pmos), a single stranged INA primor (5) mid. sotides, corresponding to the region spanning from part of the variable region (113-119 amino adid sequence in Sequence Disting No. 5' to the constant region which is common to all humanings (25 pmpl (Nipleic Arids Research 14 1779, 1984), poly C as a primer for amplifying cONA encoding variable region of the light chain (4) pmpl;, a single stranded DNA primer (21 nucleotions, corresponding to the region spanning from J region of human .kappa. chain (117-114 amino abid seguence of Sequence Listing No. 6) to the constant region (The Tournal of Biological Chemistry 157 1516, 1982; Tell 22 197, 1981) (40 pmp) , 10.times.PCR buffer 100 mM Tris-hCl, cH -... 500 mM potassium oblorade, 15 mM magnesium onloride, 5.10 (w/v) delatin (10 .mg. 1), 10 mM 4 dNFF -2 .mu.1 Takara Snurp , and Tag DNA polymeraso (2.5 40) Takara Shuzo)), and further water to make a final volume of 100 .ma.l. Thirty cycles of incubations at 94.degree. C. for one minute (denaturing step) at 55.degree. C. for two minutes (annealing step) and at 72.degree. C. for

- three minutes (elongation step) were conducted and further incubation at 72.degree. C. for seven minutes was added. Reaction mixture was subjected to otherel precipitation, and resultant precipitates were dissolved in water (30 .mu.l).
- 61 To the aqueous solution were added Klencw fragment [2 U] (Takara Shuzo), 1 mM 4 dNTP (4 .mu.1), and 10.times.blunting buffer (500 mM Tris-Hol, pH 7.6, 100 mM magnesium obloride) (4 .mu.1), 4% .mu.1 in total, and the mixture was allowed to react at 27.degree. 3. for 30 minutes to obtain a double-stranded cDNA naving blunt ends.
- 62 b. Determination of pase sequence of cDNA
- The cDNA solution obtained above was subjected to 2 agarose electrophoresis, and a band was observed at about 500 kp. The band was our away from the agarose yel. The cDNA was inserted into a cloning vector pDCLLB at Smal site, and the base sequence was determined by dideoxy method, which revoked that among total base sequence of the PCR fragment, the base sequences encoding variable regions on the reavy and light chains were respectively those shown in Sequence Listing thes. Band 4.
- The amino acid sequences of variable regions of heavy and light chains of antibody SAH produced by the above-mentioned hybridoma were deduced from the base sequences determined acove and are respectively shown in Sequence Listing Nos. I and 6. Based on the base sequences determined, antibody GAH was shown to belong to IgG1 subclass. The DNA fragment, the base sequence of which has been determined, can be prepared by means of DNA synthesizer with good reproducibility, and therefore, the acquisition of the DNA fragment does not require the repetition of the above procedure.
- 65 EMAMPLE 4
- 66 Extablishment of Human Monoplonal Antibody 1-3-1 Producing Hybridona by Cell Plains between Lymphopyte Derived from Cander Associated Lymph Node and Mouse Myeloma
- 67 .1) Preparation of Lymphocyte
- In substantial accordance with the procedure described in Example 1 (1), tympologytes (3.times.10.sup.7 were prepared starting from bander associated lumph node extracted from a patient with lung cancer.
- 69 for Cell Pusion.
- 20 Eymphocyte cells obtained above were fised with mouse myeloma cells (%.times.10.sup.6) using polyethyleneglycol (Boehringer-Mannheim) according to the conventional method. In the same manner as Example 1 (%), the fused cells were suspended in BAT addition medium to obtain cell density of 1.2.times.10.sup.5 and and planed on a 96 well plate at a ratio of 100 inc.1/plate. Half of the culture medium was substituted with BAT addition region from time to time and the culture was continued intil hybridoma's colonies appeared. The hybridoma's colonies were observed in all of the wells. In the same manner as in Example 1 (2), the supernatant of the culture in each well was tested on the reactivity to fixed cancer cell lines such as colon rancer cell line C-1 and stomach cancer cell line MRN45, in accordance with the procedure described in Experiment 1 (2)-a. Positive wells were 16.3% (94 well) against C-1 and 6.3% (36 wells' against MEN45, and 4 wells showed positive reaction to both lines.
- 71 Cloning of hybridoma cells was conducted using the wells which showed positive reaction to both lines. The cluning was conducted three times by means of limiting dilution method, and hybridoma clone 1-3-1 was established.

- 72 EXAMPLE 5
- 73 Furification and Labeling of Monoclonal Antibody 1-3-1
- 74 (1) Culture of Hybridoma 1-3-1 and Purification of Monoclonal Antibody 1-3-1
- For culture of hybridoma 1-3-1, eRDF culture medium (Gokuto Seiyaku) to which 3% of the serum described in Example 2 (1) had been added was used. The culture of hybridoma 1-3-1 was then charged into a Protein A-agarose column, and adsorbed antibody was then eluted but to obtain purified antibody 1-3-1. The antibody was continued to be a pure IgM by SDS-PAGE.
- 76 (3) Fluorescent Labeling of Antibody 1-3-1
- 77 The purified antibody 1-3-1 was labeled by FITC according to the method described in Example 2 (2). Absorbance of fractions containing labeled antibody was measured at 00.sub.28 cm and 00.sub.495 nm, and labeling degree was determined. S/P ratio was 6.1.
- 78 EKPERIMENT 4
- 79 Study on Reactivity of Human Monopolonal Artibody to Cancer Cell Lines.
- 80 (1) Cancer Cell Lines and Preservation thereof
- 81 Himan bolon bander bell line Q-1 and stomdon bander bell line MRN4t were preserved and grown at 37.degree. C. and % 20.sub.2 bonditions in Culture Medium B in the same manner as described in Experiment 1 (1).
- 82 (3) Study on Reactivity to Living Cancer Gell Lines
- Cancer cells were cultured in a flask or Petri dish and culture supernatant was discarded. To the residue was added PBS solution containing 0.02% EDTA, and the mixture was left to stand at room temperature for 31 minutes allowing the cells to float. The ceils were washed with Culture Medium B by centrifugation and suspended in BBS so as to make the cell density of about 1.times.11.sup.6 /200 .mu.l. Antibody 1-3-1 obtained in Example 5 (1) was added to the above solution to make the final concentration of the antibody of 10 .mu.g.ml, and the mixture was allowed to react at 0.degree. C. for 60 minutes. The suspension was centrifuded at 2011 rpm for 1 minutes and the supernatant was discarded. To the remaining bells was added FITC labeled anti-human antibody solution (200 .mu.l) (Capel diluted with 18 BSA-rontaining 983 by SII times, and the resulting deil suspension was Kept at lidegree. C. fir 6) minutes. The suspension was centrifuged at 2010 rpm for 2 minutes to remove the appercatant, and the remaining cells was suspended in and washed with PBS (1 ml) by centrifugation, and the bells were finally suspended in FBS (301 .mu.1) containing PI (10 .mu.p/ml). The resultant cell suspension was subjected to FCM, and magnitude of fluorescence (FITC and PI) bonded to particular cell was determined. The rescribities of antibody 1-3-1 to polon pandar cell line C-1 and stomach cancer sell line MEN45 are respectively shown in FIGS. 3 and 4 of the accompanying grawings. In the figures, the abspissa shows fluorescence intensity per cancer cell and the ordinate shows the number of the cancer cells. As a control, a commercially available IgH antibody (Capel) was used, and the reactivities of the IgM antibody to the above-identified cancer cells were determined. In the figures, the dotted line and solid line show the reactivities of antibody 1-3-1 and the control respectively. These figures show that antibody 1-3-1 has a strong binding ability to cancer cells.
- 84 EMPERIMENT 5
- 85 Reactivity of Human Monopolonal Antibody 1-3-1 to Cells Derived from Fresh Canter Tissue and Non-Canter Tissue

- In order to study a binding specificity of antibody 1-3-1 to cancer cells, normal cells were simultaneously isolated from fresh tissue belonging to the same organ of the same patient, from which cancer cells were obtained, and reactivities of antibody 1-3-1 to respective cells were measured. Isolation of cells from the tissue was conducted according to Tokita's method as described in Experiment 3.
- The reactivity to the colls obtained above was determined by FCM in the same manner as previously described in the living cancer cells. However, the cells were washed with Culture Medium B, sispended in serum derived from healthy volunteers, which serum contained fluorescent labeled antibody 1-3-1 (final concentration of 50 .mu.g/ml) prepared in Example 5 (2), to the cell density of about 1.times.10.sup.6 200 .mu.1. The suspension was allowed to react at C.degree. C. for 60 minutes and subjected to centrifugation at 2000 rpm for 2 minutes to renove the supernatant. The remaining cells were suspended in PBS (1 ml) and washed by centrifugation. The cells were resispended in PBS (300 .mu.l) containing PI ($1^{\frac{1}{6}}$.mu.grml), and the suspension was subjected to FCM. The amount of flugrescent (FITC and PI) ponted to a particular cell was measured. Markers (b species) for determining the amount of fluorescence (quantitative kit as previously prescribed) were subjected to FCM inder the same condition. Average impunt of FITT bonded to a single cell was calculated. Based on the average amount and SVP matic of labeled antibody calculated in Example 5 $\pm \tilde{z}$), the average number of antibodies bonded to a living pancer cell was calculated. The results are shown in Table 4.

TABLE 4

St. Smath Canber Non-dander Canner Non-dander Ant ibody Cells ?∈11s -Cella Cella 7.5 .times. 19.sup.4 1-3-1 1.04 .times. 11.sup.4 1.8 .times. 10.sup.8 0.03 .times. 10.sup.3 Control 0.18 .times. 11.stp.4 0.04 .times. 10.sup.4 0.2 .times. 10.sup.3 0.3 .times. 10.sup.3

- The reactivity of the human minoplonal antibody 1-3-1 to non-cancer cells was the same level as, or loss than, that of the antibody which was derived from peripheral blood of healthy volunteers and fluorescent-labeled in the same manner as antibody 1-3-1, while the average number of antibodies bonded to cancer cells is remarkably higher than that in the non-cancer cells. In addition, the number of antibodies bonded to cancer cells was 11 times greater than that in the control antibody both in stomach and colon cancer. These results indicate that antibody 1-3-1 recognizes an antigen dominantly expressed on the surface of cell nemorate of cancer cells.
- 89 EXAMPLE 6
- 90 (1) Determination of Subplace of Light Chain of Monoplonal Antibody 1-3-1
- 91 In order to determine the subclass of the light chain of antibody 1-3-1, the same procedure as described in Example 3 was repeated except that antibody 1-3-1 obtained in Example 6 (1) was used in place of antibody 3AH. The light chain of antibody 1-3-1 reacted with anti-numan clambda, chain antibody, which was detected through the appearance of coloured band. This revealed that the

- light chain was .lam!da. chain.
- 92 (3) Preparation of Gone Encoding Monoclonal Antibody 1-3-1 and Determination of Base Sequence
- 93 a. Preparation of cOMA encoding antipody 1-3-1 by means of PCR
- Appending to the method detailed below, poly(A) containing RNAs were prepared from antibody 1-3-1 producing hybridoma obtained in Example 4 (2) using dishidine thicographs—lithium obloride method (DNA = 3.09, 1983).
- In the same manner as described in Example 3 except that the number of hyperidoma colls used was 2 times. II. sup. 6, the mEMA was prepared. The resultant EMAs (about 1.8 mg) was dissolved in a solution of roll dimprising 11 mM tris-HCl, pH 8.1, and 1 mM EDTA. A 230 .md. I aliquat of the solution provided about 10 .md. g of mEMA containing poly(A) after purification by means of mEMA FURIFICATION KIT (Pharmacia). Following the productive described in Example 3, the poly(A containing mEMA (4.8 .md.g) was dissolved in water (10 .md.l), and to the solution were added blight (T 12-18 primes (5.6 .md.g), 10 mM 4 dNTP (2 .md.l), reverse transtriptase 40 Te, EMase inhibitor 36 U), 5 times reverse transcriptase buffer 6 .md.l), and additionally water to make a total volume of 30 .md... The mixture was allowed to react at 4 .degree. C. for one nour, followed by ethanol precipitation to obtain DDMA.
- The cDNA thus obtained was dissolved in water (20 .mu.l). To the solution were added a 5. times.terminal depxyndolectide transferase buffer (5 .mu.l), terminal decxynuplectide transferase (11 T), and 10 mM dGTP (1.5 .mu.l) to make a total reduce of .5 .mu.l by adding water (6.5 .mu.l), and the mixture was allowed to respond to 5 .degree. The for 1 near to add poly d(GT at all terminal of dDNA. After templeation of the reaction, the enzymes were inactivated by heating at 1 .degree. C. for 11 minutes.
- 97 First was disclosived using the cONA thus obtained as a template. Thus, to the above reaction mixture (2.5 .mu.l. were added, as a primor for amplifying cONA encoding variable region of the heavy chain, poly C '14 nucleotides) which hyperfores dG tail added to 3' terminal of the cONA 25 ymol), a single strander ONA primer '17 nucleotides' corresponding to the base sequence of constant region of LyM shown in Sequence Disting No. 7 (.5 pmol) (Nucleic Acids Fraser th 1: 4278, 1960), poly C as a primer for amplifying CONA encoding variable region of the light chain (30 pmol), a single stranded ONA primer (19 nucleotides) (25 pmol) corresponding to the base sequence of constant region of lambous chain, shown in Sequence Disting No. 8 (Nuture C94 836, 1981). The mixture was treated in the same manner as described in Enample 3, which provided a double-stranded cONA naving blunt ends.
- 98 : Determination of pase sequence of cDNA
- 99 The DDMA solution obtained above was subjected to a marrose electrophoresis, and a band was observed at about 100 bp. The band was out away from the agarose pel. The DDMA was inserted into a clining vector pDDLL9 at Smal site, and the case sequence was determined by didnoxy method, which revealed that among total case sequence of the PDR fragment, the base sequence encoding variable regions to the reavy and light chains were respectively those shown in Sequence Listing Des. 9 and 1.
- The amino acid sequences if variable regions of heavy and light chains of articoly 1-3-1 produced by the above-mentioned hybridoma were deduced from the base sequences determined above and are respectively shown in Sequence Listing Nos. 11 and 12. The DNA fragment, the base sequence of which has been determined, can be prepared by means of DNA synthesizer with good reproducibility, and therefore, the acquisition of the DNA fragment does not require the repetition of the above procedure.

- 101 EXAMPLE 7
- 102 Preparation of Adriamycin-Containing Liposome Bonded to Antibody GAH
- 103 a. Preparation of Thiolated Antibody
- Anti-cancer antibody GAH (InG) was disselved in 0.1M--acetate buffer (pH 4.0), and pepsin (1/41 mol) (Gooper Biomedical) was added thereto. The mixture was allowed no react overnight to prepare F(ab').sub.2. Chromatography over dation-exchange resin (mont S) (Enarmadia' isolated F(ab').sub.2. The solvent used was a linear gradient of 6.1M--acetate buffer (pH 4.0) containing 0-0.6M NaCl. In the isolated F(ab' .sub.2 in 1.1M--acetate buffer (pH 4.0) containing 0.18M NaCl was added DTF at a ratio of 18 .md.1 of 10% DTT/1 mp antibody. The mixture was left to stand at moon temperature for 50 minutes. After completion of reactaon, the mixture was passed through a gel filtration solumn FD-10) equilibrated with PBS for cosalification to obtain thiolated Pab'.
- 105 h. Thislation of polyethylene glycol
- 106 L-cytteins 45 mm) was disculved in 0.4M horate suffer (10 ml), and -,4-bis polyethytene glycol -6-chapto-s-triadine (200mg) (activated PBG L) (Seikagaku Rogy) was added thereto. The mixture was allowed to readt at room temperature evenight. To the resultant PBG bonded with bysteine was added DTT (02 mg), and the mixture was allowed to roadt at 30 degree. Of for 6 hours to obtain a solution containing PBG bonded with bysteine. The solution was gel filtrated (GB-35, Seikagaku Rogyo) for desalting, and the solvent was substituted by 10 mM phosphate buffer (pB 7.4) and 0.15M-NaCl (PBS). The solution was added to thiopropyl Sepharose (B (Eharmadis) equilibrated with PBS, and non-bonded substances were washed away by PBS. Cysteine-pinding PBG adsorbed to the gel was eluted out by PBS bont airling 80 mM-DTT, which was then subjected to gel riltration to remove (xpession DTT. This gave thielated PBG.
- 107 of Maleimodation of appalmatcylphosphaticylethanolamine
- Dipalmitoylphosphaticylethan:lamine (127 mg),
 N-(.epsilon.-maleimicocaproyloxy)scottnimede (EMCS) (81 mg), and triethylamine were added to a chloroform/methanol (5:1) solution (44 mg.l), and the mixture was allowed to react for 3 hours under nitrogen gas. Additional EMCS (20 mg) was laded and the mixture was allowed to react at room temperature for further hours. After confirmation of negative numbraria reaction of the reaction nixture, the mixture was evaporated to dryness under reduced pressure and the residue was dissilved in a trace amount of chloroform. The maleimidated dipalmitoylphosphaticylethanolamine thus obtained was purified by chromatography over UNISIL (Gasukuro Rogy) equilibrated with chloroform, using a chloroform/methanol (16:1) solution as an elucot.
- 109 a. Fregaration of liposome containing admismyoin bearing maleimide group
- Jolia lipid mixture (100 me. 'Mippon Seika), which consists of dipalmitoylphosphaticylcholine (DPPC), cholesterol (Chol), and maleimidated dipalmitoylphosphaticyletharecamine at a ratio of H:H:C.3 (mol) was added to 1.3M citrate kuffer (pH 4) of ml) and admixed. Freezing and thawing of the mixture was repeated 1 times to achieve hydration. This gave multimera liposome. The lipisome was marged in an extruder (Lipeltimes. Signembranes) equipped with a polycarbonate membrane (Nooleopire; Microscience) having a pore size of .Cl nm and kept at of degree. C. Repeated pressure-filtration (10 times) gave a dressed lipic mod. The liposome solution was neutralized with addition of IM NaOH solution, and to the neutral solution was added one tenth by weight: b: additionycin (Kyowa Hakko) with respect to the lipid components unile being cept at & degree. C. More than 97% of additionycin was positively enclosed into the liposome according to the pH slope between the chaide and outside of the liposome to give a liposome into which additionycin bearing maleimide group had been encapsulated.

- 111 e. Binding of maleimide group-bearing adriamytin-encapsulated liposome to thiclated antibody and PEG modification
- 112 To the adriamycin-encapsulated liposome obtained above (lip.d components: 100 mg) was added thiblated Fap' antibody (5 mg), and the mixture was allowed to react at 37.degree. C. for 8 hours. To the reaction mixture was added thiolated PBG (6 .mg.mol), and the mixture was allowed to react in PBC at room temperature for ϵ hours to obtain admissible encapsulated 1.posome bonded to antibody and moditied with PBG. The latter was further subjected to del filtration using SEPHAROSE C16B (Pharmadia; "SEPHAROSE" is a redistered TM for ion exchanged: to remove non-reacted cysteine-binding PEG.
- EXPERIMENT : 113
- Confirmation of Fharmaceutical Effectiveness of Adriamygia-Engageulated Liposome Formald t Antibody SAH and Modified with PBG
- 115 Study to anti-tanier effect of antibody SAH was conducted it the manner as described keliw using numan stomach bander bell line MKN45 union had snown reactivity to antibody GAH and accumulative behavior in transplantation to nude mouse.
- 116 Cultured MENAT cells (1.times.10.sup.6) were subputaneous-transplanted to nude mouse. Experiment started when the bunder weight became about 110 .mu.g after ten days from the transplantation (FIG. 5). Adriamydin-endapsulated liposome bonded to antibody GAH and modified with PEG was administered to mouse via datable wern et a bose corresponding to E mg/kg or adriamydic day 0, 3, 7 (shown by mark .minming. in PIG. 8). As a control, phosphate puffered physiological saline shown by mark diamond-solid.), adriamydin (shown by mark .quadrature. , and adriamy in-encapsulated liposome modified with PEG (snown by mark times. were administered to mice (each 6-7 animals). Time-course measurement of prowth of cancer was conducted by means of Battle-Columbus method unerein presumptive pancer weight was determined addording to the formulation: (short diameter).times.(short diameter).times. long diameter)/2, and compared with that determined at the beginning of the emperiment.
- 117 In FIG. 1, the abscissa shows time-lapse (days) after beginning of the experiment, and the mark (.dwnarw.) indicates the administration of the pharma multimal formulation of the invention. FIG. 5 clearly shows that the formulation of the invention, adriamyrin-encapsulated liposome bonded to antibody CAB, pissesses potent anti-dancer effect. It is apparent, therefore, that human repositinal antibodies of the invention allow continuous and long term "targeting therapy" of cancer tissue or organ with the help of anti-cancer agents or toxins.

SEQUENCE LISTING

- *1 GENERAL INFORMATION:
- (iii) NUMBER OF SEQUENCES: 42
- 1. The server FOR SEQ ID NO: i SEQUENCE THARACTERISTICS: A LENGTH: 3 base pairs 5 TYPE: Lucieic acid (C) STEANDEONESS: 30000 +2. INFORMATION FOR SEQ ID NO:1:

- STRANDEDNESS: double
- D: TOROLOGY: linear
- it MOLECULE TYPE: dDNA
- inth HYPOTHERICAL:
- (iv ANTI-SENUE:
- (v) FRAGMENT TYPE:
- (vi) DRIGINAL SOURCE: human Ig3 antibody
- (A) DEGANISM:

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(B) STRAIN:
(C. INDIVIDUAL ISOLATE:
(D) DEVELORIMENTAL STAGE:
(F) HAFLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(I) OFGMELLE:
·A· IIBPAFY:
GBO CT THE:
CHILL FOUNTION IN GENOME:
(A) OBECIDIONE (SEGMENT:
 BUILDING BURITION:
     ·ix) FEATURE:
 'A' NAME FEY:
ARY LOCATION:
 o menoration method:
 D. GIBER INFORMATION:
 M FURNI WILDOW IMPORMATION:
 A ATTRONE:
 sens to MEMAL:
ON WHIME:
(E) ISSUE:
JER FAGES:
GN DWTH:
THE IN COMMENT NUMBER:
     FINING DATE:
FUBLITATION DATE:

FUBLITATION DATE:

FUBLITATION DESIGNES IN SEQ ID NO:

EL) JE JEROTE DESIGNITION: SEQ ID NO:1:

GOCCOTT SETTIONAGED PAAGAGACGGTGACCATTCT37
. INFORMATION FOR SEQ ID NO:2:
(1) SEQUEDOE CHAPACTERISTICS:
(A) LENGTH: Al base pairs
 TEN TYPE: nucleic acid
 e COFAMIENMENS: diuble
    -District Off: Linear
 .i) h it mle dyet: coma
 lli HYFYTHETICAL:
 .7) ANTI-PENSE:
 ·· EFANIENT TYPE:
(vi) (FIGINAL COURGE: numan IgG antibody
GAN OFFICENCIEM:
IB) CTEANI:
POR INFINITIVAL ISOLATE:
10 OFUFIL FMENTAL SIAGE:
    HAFDOTYFE:
 BO BILLION BONGBER
BO TELLO I INE:
I BONGBER LER:
WII III BONGBER SOURCE:
 A MERARY:
 Ex. (2). (2F):
 viil) POSITION IN GENOME:
A. HE MINDME SEGMENT:
 B. MAR RECITION:
 5: 000733:
 &R* FEATURE:
 AT NAME/REF:
 B: L:CATIUN:
(C) IDENTIFICATION METHOD:
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(b) THER INFORMATION:
(m) FUPLICATION INFORMATION:
(A) ATTH (RJ):
·HO TITLE:
of a ToffElling:
(L_{\rm T}/V) L^{*}MF :
H. ISTTE:
PRO FAGES:
GG: DATE:
-H: Designed tomber:
(I) FILIDO DATE:
(7) FURLICATION DATE:
PRO RELEVANT FESIDUES IN SEQ ID NO:
(Mi) JEPTENE DESCRIPTION: SEQ ID NO:2: TOGT TOATA TOTAL TO FOR JED NO:3:
(i) JEQUEDOF CHARACTERISTICS:
(A) LED WIH: 3% base pairs
     MFF: nucleur maria
407 STEARDEDNESS: double
   Ted damW: limear
 .1) MODERNE TYPE: FONA
:iv) ADTI-SENSE:
FEARMENT OYPE:
evi) OFIRMAL COURCE:
(A) OR MANISM:
PERTAIN:
OT INCHUIDUAL ISCIATE:
FIR TENENCHMENTAL STAGE:
E HARRITEE
RE TIONE TYEE:
100 THE INFE: Hybridoma producing human
anticley WH (H OFFI LIME:
(I: OF ANELLE:
(vii: NOGFOTATE SCUBCE:
(A) DIMENEY:
(b) CLOUB:
CHLIS FORISION IN GENOME:
A THROUGHE SEGMENT:
OF MAR RESIDION:
(ix) FEATURE:
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on IDENTIFICATION METHOD:
(F: OTHER INFORMATION:
ON FURE CATEIN INSOMMATION:
(A AUTH 4.0:
(E:\ \mbox{TITLE:}
OF TOTALIST
(I - W IME:
   · F · 1 A · F 2:
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+80 Designed Dimess:
 In FILING DATE:
 TO EMPLICATION DATE:
 E) RETEVANT RESIDUES IN SEQ ID NO:
(Mi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
CHGGT 3 1A 3 0 F 30 AGGA 3T 03GG 0CCAGGACTGGTGAAGCCTTCA 4 5
\texttt{CAGACO} : \texttt{TSTCOCTCACOTGCACTGTCTCTGGGTGGCTCCATCAGC90}
A FITGI JGTTT DTACTGGAACTGGATCCGCCAGCACCCAGGGAAG135
GUCCTGGAGTGGATTGGGTACATGTATTACAGTGGGAGCACCTAC180
```

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TACAACCGGTCCCTCAAAAGTCGAGTTACCATATCGCTAGACACG225
TOTAAGAGCCAGTTCTCCCTGAAGCTGAGCTCTCTGACTGCCGCG270
GACAGG SOC STGTATTALT STGGGAGGTCTACCGGAGTACGGGGGG3 1.5
É DIGACITAC PEGGECCIA POPAACAAITGGICACCCITCITCA 357
(2) INFORMATION FOR SEQ ID NO:4:
(1) SEQUENCE CHARACTERISTICS:
(A) LEDOTH: 341 base pairs
(F) TYPE: nubleic acid
 D: STRAMLEDNECO: double
(I) Torology: limear
(11) MOLECULE TYPE: CONA
 :11) HYP THETICAL:
A OF WALLSM:
   STFARD:
 D: INCOMINAL ISOLATE:
/ DETELOPMENTAL STAGE:
   HAFLETYFE:
  TIZZTE TYFE:
    THIL IME: Hypridems producing human
antir by Will
H. OBLL 1708:
 I - CFRANENIE:
(vli) IMMEDIATE SOURCE:
(A) LIBRARY:
(E) CLOUE:
(viii' losition in genome:
VA: CHECADOME SEGMENT:
OF MAR PRAITION:
(IX) FEATURE:
GROWN REFE
(E) OTHER INFORMATION:
(M. FUBLICATION INFORMATION:
(A) ADTHORM:
(A) TITLE:
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   1....F:
   EA HS:
GATE:
THE DESIGNATION OF THE EAST
(I' FILIDS DATE:
AUT POPLICATION DATE:
F RELEVANT RESIDUES IN SEQ ID NO:
MI) REQUENCE DESCRIPTION: SEQ ID NO:4:
sacarn i atraacceaafter scagactoccreectgtgtgtctctg45
GROGARM PROCESSATIVAACTGCAAGTCCAGCCAGAGTGTTTTA90
DACAA DE ENANAATAARAAATACTTAGCTTG STACCAGCAGAAAA 135
CINAGRA MARCHEROTTAAR OFFICITICAT FTA OTFISE GCATOTA OCCIGISES
GAAT OCH FRYTOCOTIGA EUBAT FOAG FIGE CAG OEGGTOTIGGGACA225
GAUTTUM ITHUGAUGAII DAOGAGUGI GCAGGUI GAAGAIIGTIGGICA270
STITATTATT ST MAS MARTALASTA SIGSST 3GAGGTTC 3 3C315
CAJAG 3 SAL YOMAN STERNAMAN DOAGAG 3A 34 2
AND INFORMATION FOR SEQ ID NO:5:
 : SF. MENOF CHARACTERISTICS:
(A) LETTER: 110 amino acids
(B) TYPE: Whine acid
 1: SIEANDEDNESS: single
(D) TOROLOWY: linear
(ii) MULECULE TYPE: protein
```

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iii, HYPOTHETICAL:
(iv) ANTI-SENSE:
(v FRAGMENT TYPE:
(VI) (PIGINAL SOURCE:
    ECANISM:
+A^+
(B STEAIN:

(C) INDIVIDUAL ISOLATE:

(C) CEVELOGMENTAL STAGE:
F HAFLOTYPE:
    TIBRUE TYPE:
   EII IVEE: Hybridoma producing human
. . .
antibody GAH
-H TELL LIME:
   - REMOTELLE:
(VLI) IMMEDIATE SOURCE:
AT TERARY:
 i Tene:
 TLILE E SITE N IN GENOME:
 A THEODOLOME (SEGMENT:
 HOLDER CHESSE
BOSTOTON:
    "DIT.":
 ix) FEATTEF:
 A DAME REY:
 {\tt B} = {\tt IOTATION};
    IDENTIFICATION METHOD:
    OTHER INFORMATION:
 E' FUELLCATIAL INFORMATION:
 AT ATTHURS:
    TITLE:
 13,5
 TBY TITLE:
| OT | MOTENALE:
·D DOMEST
    10000
    EASE.:
    FILE:
 H ICCURENT DUMBER:
 I FILING LATE:
ATH FUBICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
(MA) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GlnValGlnLeuGlnGluSerGlyProGlyLeuValLysProSer
```

```
1:1015
{\tt GlnTr.} : {\tt Lor.Ser} \: {\tt LeoThrCysThrValSerGlyGlySerIleSer}
2.-253:
SerCys 31yFheTyrTrpAsnTrpTleArgGlnHisProGlyLys
314049
GlyLeuS. TrpfleGlyTyrIleTyrTyrSerGlySerThrTyr
TyrAsnEr SerhooLysSerArgValThrIleSerLeuAspThr
SerbyuSe: SimPacSerLouLysLeuSerSerLeuThrAlaAla
AppThrAlaValTy: TyrCysAlaArgSerThrArgLeuArgGly
9510-1
A.aAspTy:TrpGlySlnGlyThcMetValThrValSerSer
110115
    INFORMATIAL FOR SEQ ID NO:6:
    JEPURNOR MARAGRERISTICS:
(A- LENGTH: 114 amino acids
(B) TYFE: amin acid
(C) STRAMBEDNEDS: single
```

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.n. TOPOLOGY: linear
 (.i) MOLECULE TYPE: protein
 (iii) HYPOTHETICAL:
  17) ANTI-SENSE:
 .v FRAGMENT TYPE:
 TVIF GRIGINAL SCURCE:
 A OFCANISM:
  P FIRAIN:
          INTIMICUAL ISCLATE:
   I - UENTELDEMENTAL STAGE:
         HARDSTYPE:
TILLYTE TYPE:
PELL TYPE: Hybridoma producing human
antirop AH H OF DUINE:
       OF WANELLE:
   THE MINE CATE SCURCE:
   A NIERAET:
        · 11 · 11 · 1
   WALL BESTITION IN GENOME:
   A THE CONTROL OF CMENT:
   F IME & CITION:
   ix) FRATURE:
   A MANE FEY:
  P \in L^p(MTI)(\Omega)
  OF IDENTIFICATION METHOD:
   to OTHER INFORMATION:
  E POPULTATION INFORMATION:
   A ACCEMENT
         Piche:
         17. TF:
 IF PARE:
         DATE:
 THE DESCRIPTION NUMBER:
 'I' FILIDS LATE:
 OF PURLICATION DATE:
 THE RELEVANT FENIOUES IN SEQ ID NO:
  MID DEPOSE TWEETERS ON SEQ ID NO:6:
Ampile Taller ThankserEmtAspSerLeuAlaValSerLeu
 1 1)1.
GlyGlaA: :AlaThrlleAsnCysLysSerSerGlnSerValLeu
202531
TyrAsmCerAsmAsmbysLysTyrLeuAlaTrpTyrGlnGlnLys
 2-4045
ProGlyWinFreProbysLeuLeuIleTyrTrpAlaSerThrArg
5.556
GluSor LyttalProAspAngSheSerGlySerGlySerGlyThr
AppProlimination of the AppProximation of th
 ValTyrlyr TysGinGlaTyrTyrSerThrProTrpThrPheGly
 954001.
GlnGlyTrallysValGluIleLysArg
           IMP SMATION FOR SEQ ID NO:7:
           SEPTEMBER CHARACTERISTICS:
           LED CH: 17 base pairs
            IFFE: nucleus asia
         B:BANDHDNEJ.: doub.∈
   D) DJPDL HY: linear
   ii) MOLE TULE TYPE: dDNA
 (iii) HYPOTHETI AL:
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+i*: ANTI-SENSE:
/v FRAGMENT TYPE:
(71) RIGINAL SOUPCE: human IgM antibody
(A) = O(A)HSM:
(P) 3TFAIN:
(C) IMPINITUAL ISOLATE:
(D) DEVELORMENTAL STAGE:
(E) HAILOTYPE:
(F TIESTE TYPE:
(A) CELL TYPE:
(B) CELL LINE:
(I) OBGAUELNE:
HOLIT IMMEDIATE S.URCE:
A DIFFREY:
...ii | FOSITION IN GENOME:
AND THEAM WAIME FE MENT:
OF MAR E SITION:
(1x) FFATTEE:
RA SMATE PEY:
* ON OTHER INFORMATION:
** FURNITATION INFORMATION:
A. ANTHOEN:
(D) DOUBDAL:
FEL TRAME:
FEE EWEEN:
POST DESTRICT
   DO THE STORMER BY
BUILDING DATE:
BUBLINATION SATE:
(F FELEVANT RESIDUES IN SEQ ID NO: (%1: SEQUEDOE DESCRIPTION: SEQ ID NO:7:
CGAGG 3-30MMMAGGGTT 1.7
(I' INFORMATION FOR SEQ ID NO:8: (E' SEWMENCE CHAFACTERISTICS:
   LENGIH: 19 base pairs
(B. TYFE: nucleic acid
    278ATTRIMEDE: double
T.FOIR OF: linear
FILE HELE THE TYPE GONA
(mai/ HYFCTHETICAL:
(17) AND 1-SENSE:
(W) FRAGMENT TYPE:
(VI) SELGIMAL SOURCE: human IgM antibody
(A) OF MALICH:
· B STRAIN:
   INCIVICUAL IS LATE:
CHUEL HIENTAL STAGE:
** BUILDIEPE:
VEN TINUTE TYPE:
(3 DELL TYPE:
08 DELL LINE:
   ORGANELLE:
···.i` IMMFDIATH SOURCE:
 A DIREMET:
 B Charles
 "HIL HONITION IN GENOME:
 A CHROME SEGMENT:
, BE MAP POUTION:
(C) UNITS:
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(ix' FEATURE:
(A) NAME 'YEY:
(B) LOGATION:
(C) HIENTIFICATION METHOD:
(C) OTHER INFOFMATION:
(x) FUBLICATION INFORMATION:
(A) ASTHORA:
(E) \in \texttt{TITLE} :
-år formuål:
-D- vileme:
F: 12.77
 F. EART.
    H DOUBLE WINDER:
*I * FILLUS PATE:
    ETENNATI O DATE:
FY RELEAVANT REGIOUSE IN SEQ ID NO:
x1) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GAAG HOUSEWAA PAGGGT)
     INFORMATION FOR SEQ ID NO:9:
 TO MELTERNO CHARACTERISTICS:
 A LEDGTH: 300 base pairs
F) TYPE: nucleic sita
() STPANGENMESS: diuble
CO TOFOLOGY: linear
HILL HYPOTHEFICAL:
 iv) ANTI-SENSE:
 # PRACTIENT TYPE:

HI) OBIGINAL SOURCE:

A GCANTSM:
# STEAIU:
07 INDIVIDUAL ISCLATE:
00 DEVELOPMENTAL STAGE:
AE HARLHIYEE:
FF TIRRUE TYPE:
OF TELL TYPE: Hybridoma producing human antibody 1-3-1
THE PELL DINE:
:I SHAMBLLE:
(vii) HIMBUATE SOURCE:
(A LIBBARY:
.vii: 103171-11 IN SHNOME:
(A THEOMS SOME SEGMENT:
(B MAR FOSITION:
(C MARCE:
+1x) FEATURE:
(A MAME EFY:
(\mathbb{R}) LOCATION:
OT IDENTIFICATION METHOD:
OR FURIL MATERN INFORMATION:
A ATTEORS:
   TITLE:
FOURDAL:
(D. VOLTIE:
(E. ISSTE:
(F. PARE:
    1957 F.:
    DOTTHENT WINBER:
 1. FILING DATE:
 TO PUBLICATION DATE:
 K) RELEVANT REJIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
CAGCT 3CA 3 2T 3 2A 3 3A 3T 25GGCCCAGGACTGGTGAAGCCTTCG4 8
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GABACCTGTCCCTCACCTBCACTGTCTCTGGTGGCTCCATCAGC90
AGRAGIAGTTACTACTGGGGGCTGGATCCGCCACCCCCCAGGGAAG135
GUAGOTA JAGTUAGATTIGG BABITATICTA PTA LAGTIGGGAGICACOTACI 80
TABAAB NOGTOONTOAA NAGTOGAGTOACCATATOÙGTAGAGAGAGG2225
TO CAAGAACCA GOTOCO TGAAGCTGAGCTCTGTGACCGCCGCA270
GACA DE SOT STIGTATTAC E STIGOGAGIGGGGAGCTACGGGGGGCTACC 15
TACTAC SGTATGGACGTOT SGGGGCCAAGGGACCACGGTCACCGTC3 60
TOCTCA366
 (a) INFORMATION FOR SEQ ID NO:10:
 (:) SEQUENCE CHARACTERISTICS:
 (A) LEMPTH: 334 base pairs
  The TYPE: nacheit stid
 or strandedNads: double
+10 PQEOLOGY: linear
+1i) MULFOULE TYPE: 50NA
+1ii) HYPETTETICAL:
  · : 7) ANTI-SENSE:
 · · · FÉAGMENT TYPE:
 eri) ARIGINAL SOUFFE:
   Ar OF WOLLTM:
   Bar BIBLID:
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   E) HARIOTYPE:
  FI TIESUF TWEE:
 .G) CELL TYPE: Hybridoma producing human antibody 1-3-1 _{\rm cH} CELL LINE:
    I - GERMHELLE:
   vii) IMMEDIATE SOURCE:
   A: DIFFAFY:
   5 ( CERME:
   THIS BUTIFIED IN GENOME:
   AR THREAT COME SEGMENT:
   BE MAR FENITION:
   ix) FEATTHE:
   A. SAME PRY:
   Brooks Island
   OF IDENTIFICATION METHOD:
   ( ) OTHER INFORMATION:
 (x) FUBLICATION INFORMATION:
 (A) AUTHORD:
 (B) TITLE:
 (C) JOYFNAL:
 (D) VOLTME:
  EL ISSTE:
 FF FARE:
 of CATE:
   ELO DO MIMENT NUMBER:
    I - FILIM, DATE:
     JE EUBLICATION DATE:
   R) REDEVANT RESIDUES IN SEQ ID NO:
   ki) sequence description: seq id no:10:
TATGAG TIGA LACAGO DAGGOTOGGTGTCAGTGTCCCCAGGACAG45
ACGGCCAGGATCACCTGCTGGAGATGCATTGCCAAAGCAATAT90
GOTTATT SETACIAGOA SAA SOCAGGOCA GECCCCTGT SCTGGTG1 35
A TATATAA SA DA SE SA SA SECECTCAG SGAT COOTGA ECSATTC1 30
TOTGOOT COA FOTCA SG SA CAACAGT CAOSET GAOCAT CA STGGA2 25
 GT DCAG BDA BAAGA DGA GGCTGA DTATTACTG TCAATCAGCAGA D2 70
 A 3 CA 3T 3 3TA CTTATGA 3 3TATTCGG CG 3 AGGGA CCAA 3CTGA CC 3 1 5
```

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GTCTTAGGT324
(2 INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LEMCTH: 1.2 amino acids
#BY TYPE: amiro anid
(C) STRANCEDNESS: single
(f) TOFOLDGY: .inear
(11) MOLECULE TYPE: protein
(111) HYPUTHETICAL:
 IV: ANTE-SENCE:
 TERROMENT TARE:
 vi, osggnal votace:
A chcanism:
A COMMANDA

OF COMMAND ESCLATE:

OF DEVELORMENTAL STAGE:
(EI HAPLOIYPE:
(F: TIRSUE TYPE:
G: (FIL TYPE: Hybridoma producing human antibody 1-3-1 H, CFIL LINE:
    OPHREIDE:
 "LI IMMEDIATE SOURCE:
 A TIPEARY:
 F COLNE:
 TLIFE POSITION IN PENOME:
(A) CHEOMOSOME SEGMENT:
 E' MAP POSITION:
CO PRITS:
-ix) FEATURE:
A NAME KEY:
 \underline{\underline{\underline{}}}_{L}=\{(\underline{\underline{}}_{L})_{L},(\underline{\underline{}}_{L})_{L},(\underline{\underline{}}_{L})_{L}\in\underline{\underline{\underline{}}}_{L}\}
    IDENTIFICATION MERHOD:
 DOWN THER INFORMATION:
 E ESERCATION INFORMATION:
 A ATTHORA:
E TITLE:
     DOUBLIAL:
 5 VOLUME:
 E - ISSUE:
 E's EASTERS:
 G. FATE:
   - FOUNDAMENT DUMBER:
    FILING DATE:
 I FUBLICATION DATE:
PO RECEVANT FEBILITES IN SEQ ID NO:
 MIN SECTEMBE TESTIFITION: SEQ ID NO:11:
GlnLenGlnLouGlnGldSenGlyProGlyLeuValLysProSer
151015
GluTm: let3erLetThrCysThrValSerGlyGlySerIleSer
∄ 125 vi:
SerSerSerTyrTyrTrrGlyTrpIleArgGlnProProGlyLys
3:4049
G.yLelfluTrpIleGlySerIleTyrTyrSerGlySerThrTyr
TyrA. htr:CerLettly:SerArgValThrIleSerValAspThr
SerbysAsmGlnPmeSerLeubysLeuSerSerValThrAlaAla
8785.00
AspTt.:AlaValTy:TyrCysAlaArgGlySerTyrGlyGlyTyr
9510:des
TyrTyr::lyMetAsp7alTrp3lyGlnGlyThrThrValThrVal
11911:120
BerSer
(3) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
```

```
(A) LENGTH: 138 amino acids
(B) TYPF: amino acid
(4) STRANTEDNESS: single
(D) Tolk Logy: linear
(ii) MOLETULE TYPE: protein
lii) HYPOTHETICAL:
117) ANDI-SENSE:
TO FFARMENT TYPE:
(V1) ORIGINAL SOURCE:
A OF BANISM:
B ATRAIN:
    IN INIMAL ISCLATE:
   DETERMENTAL STAGE:
   HARISTYPE:
   TIBLUE TYPE:
.G. CELL TYPE: Hyperadoma producing human antibody 1-3-1
(H) CELL LIME:
,i - +4-GANELLE:
THIE HOMEDIATE SOURCE:
A I IFFAFY:
F 80000
Hell Ecological IN GENOME:
A THELLE SOME MERMENT:
RE MAR FOSITION:
(1x) FEATURE:
(A) NAME KEY:
(P) Lo MTION:
: CÖRTEM MCITACIALICA METHÖD:
(IC) (THEE INFORMATION:
EX. FUBLITATED INFORMATION:
A ANTHORS:
B: IIIIE:
CI DOBENAL:
   Valine:
   13376:
 F: FAGEA:
   DATE:
H) DOCUMENT MUMBER:
 I: FILING DATE:
T: FUBLICATION DATE:
 F FELEVAM FESIDUES IN SEQ ID NO:
(ML) PROTENCE DESCRIPTION: SEQ ID NO:12:
TyrGl::LeuThrGlr:Pr::ProSerValSerValSerProGlyGln
101018
Trankl Wirell&Tho CysSebGlyAspAlaLeuProLysGlnTyr
. 23396
AlaTyrir;TyrGlr.GlnLysPrcGlyGlr.AlaProValLeuVal
354045
IleTyrtysAsp3erGluArgPrcSerGlyIleProGluArgPhe
5.7334 -
SarGlyder SerSerGlyThrThrValThrLeuThrIleSerGly
ValGlaAla HuAspGlaAlaAspTyrTyrCysGlnSerAlaAsp
SerSerGlyThrTyrGluValPheGlyGlyGlyThrLysLeuThr
95100105
Valle 1817
 I IMPORMATION FOR SEQ ID NO:13:
 1 SEQUENCE CHARACTERISTICS:
 A- LEMPTH: 3 amino acids
 B. TTEB: amino acid
    JTFANDEDNEUS: single
D) To: ULDGY: linear
(ii) MULECULE TYPE: protein
```

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(iii) HYPOTHETICAL:
 (1v) ANTI-SENSE:
 (7) FRAGMENT TYPE:
 (71) RIBINAL SOURCE:
 (A) DE MATISM:
 (B) STRAIN:
 (C) INCIVIOUAL ISOLATE:
 (10 DEVELOPMENTAL STAGE:
 THIS HARROTYPE:
   EN TICHTE TYPE:
   IN CELL TYPE: Lybridoma producing human monoclonal
antibe by, an antiper to which exists on the surface of cancer of a memberane of the Carl Communication of the carl Commun
 culi: IMMEDIATE SOURCE:
  A) DIBEART:
 Bo Claude:
  vii., FORTION IN GENOME:
   A) THE OF BOME SERMENT:
   BO MAR ROSITION:
  n (M.)
(R) FEALTER:
  A: WAR FFT:
   Be DOTATION: 4
 (1) IDENTIFICATION METHOD:
 (E) OTHER INFORMATION: note ="Cys or Ser"
 ilk) PERTUPE:
 TAI NAME TEET:
 'En Darbatten:
 HAR INFIDITION METHOD:
  in Other Information: note ="Gly or Ser"
 +ix) FERTURE:
 FAR WALL FEY:
(B) LOCATION: (
           EFFUTIFICATION METHOD:
       OTHES INFORMATION: Onote ="Phe or Tyr"
 (E) FUBLICATION INFORMATION:
(A) ANTHORS:
(B) TILLE:
(C) MOURNAL:
17 7 LIMF:
 F ISSUE:
 · E FRED:
        DEFINITE NO NUMBER:
        FILLING DATE:
           PUBLICATION DATE:
 FF RELEVANT SECTIONS IN SECTION NO:
 (MA) SEQUENCE DESCRIPTION: SEQ ID NO:13:
IleSerDerMaaMaaMaaTyrTrp
           INFOHMATION FOR SEQ ID NO:14:
           SECHED IN CHAPACTERISTICS:
         LENGTH: 12 amino acids
          Trif: among a rid
           STEAM EDNESO: single
 Joy TOP HowY: Time ar
 (ii) MODESTURE TYPE: protein
 (111) HYPOTHETICAL:
   :v) ANTI-SENSE:
  H) FRAGMENT TYPE:
  (71) DEIGINAL COURCE:
 (A) Jk :ANIJM:
 (B) STRAIN:
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(C) INDIVIDUAL ISOLATE:
(()) DEMELORMENTAL STAGE:
HAPLTTPE:
PETILISME MYPE:
G CELL TYPE: hybridema producing human monoclonal
antibody, an antigen to which exists on the surface of
dander dell membrane
WHI CELL LINE:
( ) - OECAMELLE:
(vii) IMMELIATE SOURCE:
(A) LIBPAFY:
PARLIME:
(viii h SITION IN GENOME:
(A, -) CHECKCIOME (A, B) SECMENT:
eb ind ledition:
(IX) FEATURE:
(A) DATE KEY:
(B) DEFATION:
  CHRITIFICATION METHOD:
-D - CTHER IMPORMATION: note = "Tyr or Ser"
R FUNDICATION INFORMATION:
4A A 均标 E.3:
·B, TITHE:
(C) TOTRUAL:
(2) VOLUME:
(E) ISSUE:
(F) FAGES:
:G: CATE:
H CONTRENT TRUBBLE:
  ELLING DALE:
ENELLICATION DATE:
-K: RFIRWAMT RESIDUES IN SEQ ID NO:
(MI) SEQUENCE DESCRIPTION: SEQ ID NO:14:
lleGlyMaalleTyrTyrSerGlySerThrTyrTyr
    INFOFMATION FOR SEQ ID NO:15:
*1 SECTEMOR CHARACTERISTICS:
(A) LFNGTH: 4 amine acids
(B, TYPE: amand adid
or STRANCEDNESS: single
(D) TOPOLOGY: Danear
:11) MONGCTLE TYPE: protein
*:21 HYFOTHETICAL:
(17) ANTI-SENSE:
the BEAR MENT TYPE:
-vi) OFIGINAL SOURCE:
RAN GESAGH EIT:
HEN STEAIN:
(): INDUVIDUAL ISOLATE:
 DE CEVELOFMENTAL STAGE:
E HAPINTYME:
    MISCUE TYPE:
   THIS ITPE: hybridoma producing human monoclonal
anticody, an antiger to which exists on the surface of
cancer cell membrane
H: CELL LINE:
T: DRGAMELLE:
THE IMMEDIATE SOURCE:
(A) LIBRART:
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(B) CLONE:
("lil" POSITION IN GENOME:
(A) CHECHOSOME SEGMENT:
(B) MAI POSITION:
(C. UNITE:
(ix) FRATURE:
(A) MAME KEY:
B DECAPTON: .
    DIFENTIFICATION METHOD:
- 1
(F) OTHER INFORMATION: Inote = "Ala or Met"
Hix) FHATTES:
AT DAME FEY:
B D (WII) M: 4
    U EDTIFICATION BETHOD:
   018FF INFOFMATION: rote ="Tyr or Val"
PUBLICATION INFORMATION:
   ATHER:
F - TITLE:
   TAMEBUAL:
 THE VALUE :
   IDATE:
   195035071
    14.15:
   ICONTIENT NUMBER:
 is Fining Dark:
   TOBNICATION DATE:
E RELEVANT RESIDUES IN SEQ ID NO:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
GlyXaaAspHaa
   HIPPORTATION FOR DEQ ID NO:16:
   CHOCHUCE CHARACTERISTICS:
   -DFMOTH: 9 amino acids
    TYPE: anim a did
   JIAAN FONBUS: singl-
    The committee linear
 na) NOLECTLE SYFE: profein
mai HYFOTHERICAL:
iv) ANTI-SENSE:
(v) FRAGMENT TYPE:
vi) PELGINAL COVECE:
A FRANCEM:
 F MEATH:
    INCOMIENAL ISCLAME:
    TEMBLICEMENTAL STACE:
    HAFI: TYPE:
(B) TIMBEL TYPE:
Of CELL TYPE: Hybridons producing human antibody GAH H CFLB LIME:
  OF CANFILE:
(vai) IMMEDIATE SOURCE:
'A DIBĒĀĒT
    in other:
 with BOSITION IN GENOME:
    THEOMOSOME SE MENT:
    MAR EDGITION:
(ix) FEATURE:
A: NAME FEY:
(B) 10-0A1100:
(C) 11EUTIF1CATION METHOD:
   DIHER INFORMATION:
(k PUBLICATION INFORMATION:
(A ATTHORS:
(B) TITLE:
(C) JOURNAL:
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(D) WELUME:
(E) LISUE:
F - PAGEA:
(G) CATE:
H: D:CUMENT NUMBER:
'IN FILING DATE:
(I) FUPLICATION DATE:
HO RELEVANT RESIDUES IN SEQ ID NO:
(ML) DEQUENCE DESTRIPTION: SEQ ID NO:16:
IleSerSerByrGryPheTyrTryAsn
*I* IMPORMATION FOR SEQ ID NO:17:
.: SEPTEMOR CHARACTERISTICS:
(A) LHNOTH: 10 aming acids
ob TYPE: amino atia
   STEACHDEIMERE: single
#FT TOTOLOGY: linear
### THE TYPE: protein
### THETICAL:
·iv) ANTI-SENSE:
v - FFA MENT TYPE:
tri) CELOTHAL SOURCE:
(A) ORGANISM:
TER STEATH:
47 - INCHMINGAL ISCLATE:
 1 - DEVELOPMENTAL STAGE:
 F- HARIOTYLE:
F: TISOUE TYPE:
F: TISOUE TYPE: Hyperidoms producing human antibody GAR
H: CFID: LIDE:
 I. OF BANELLE:
THE IMPRICATE SOURCE:
A) LIBRARY:
A) CLONF:
 viii, FOSITION IN GENOME:
A CHECKEROME SEGMENT:
 F MAR EDUCATION:
 iki FEATTER:
   NAME FFY:
 E- L-DATTON:
  ICENTIFICATION METHÓD:
 IN OTHER INFORMATION:
(M) FUBLICATION INFORMATION:
AN ANTHORA:
P) TITLE:
(C) DEFINAL:
no viinas:
E - 1.78.76:
 Pro PAGE:
 PARTE:
H) OF COMPANY NUMBER:
J) FUBLICATION DATE:
H) RELEVANT RESIDUES IN SEQ ID NO:
wi) REPUBLICE DESIGNATION: SEQ ID NO:17:
IleGlyTyrL:eTyrTy:SerGlySerThrTyrTyr
1-10
(Z) [NPDRMATION FOR SEQ ID NO:18:
(i) JEQUENCE CHARACTERÍSTICS:
```

```
(A) LENGTH: 3 amino acids
 (B) TYPE: amino acid
(C) STRANTEDNESS: single
(D) TOFOLIGU: linear
(ii) MOLECULE TYFE: protein
(iii) HYPOTHETICAL:
(iv) ANTI-SENSE:
(7) FRAGMENT TYPE:
(71) (RIGINAL SOURCE:
(A) OFGANISM:
B STFATU:
00 INCLUIAN ISCLATE:
(P. DECEN EMENTAL STAGE:
           BAFICTYPE:
OFF TISHER SYPE:
OF CELL TYPE: Hybridoma producing human antibody GAH (H) CELL LINE:
 (I/ OFWANKLIE:
(MEI) HIMEBLATE SCUBCE:
(A) LIBEARY:
(B) CLOME:
chail letifient in Genome:
AA CHS-MIGONE SEGMENT:
CHS MAR FISHBODS:
AD QUART.
 *:x: FFATURE:
(A) NAME KEY:
(D) IDENTIFICATION METHOD:
(TO OTHER INFORMATION:
(E' FUELICATION INFORMATION:
A ATTHEST:
              7:7:F:
       SOME DATE:
C VETTER:
F FARES:
(3) DATE:
(B) DOCUMENT NUMBER:
 (I' FILING DATE:
OF FUEL BOATION DATE:
FROM THE PROPERTY OF THE PROPE
SerThiA: ileuArmNyAlaAspTyr
1 :
       - INPOPERATION FOR SEQ ID NO:19:
+: SEQUENCE CHAFACTERISTICS:
(A) IENOTE: 17 amino acids
 *P TYPE: amuno acid
 40 - STRANDEDNESS: single
   Do Townski nomean
   ii) MONETULE TYEE: protein
 (ili) HYBOTHETICAL:
   iv) ANTI-SENSE:
   vi FRARMENT TYPE:
   "1) BI HIMAL SOUPCE:
   A - OBTANISM:
   B. SIRAIN:
    TO INDIVIDUAL ISOLATE:
 (D) DEVELOPMENTAL STAGE:
   E - EAPL TYPE:
  FT TIBBUE TYPE:
  [3: TELL TYPE: Hybridoma producing human antibody GAH [H: TELL LINE:
 (I) DEGANELLE:
```

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(vii) IMMETIATE SOURCE:
(A) LIBEART:
(B) CLOUE:
("iii) FOUITION IN GENOME:
(A) CHE ME COME SE MENT:
(B) MAP PUBLICH:
OF THITS:
ix) FRATURE:
IA HAME BEY:
ED LOCATION:
    INENTIFICATION METHOD:
    DIRECTIFORMATION:
   EMPILMATION INFORMATION:
A ANTHORN:
    7047BINAL:
   THIE:
    TRACE:
·F PAGES:
/ N TATE:
THE COMMISSION NUMBER:
*I FILING DATE:
    FORDINATION DATE:
F RELEVANT RESIDUES IN SEQ ID NO:
MAN CELUENCE DES EXPRION: SEQ ID NO:19:
LysSerDerG.r.SerValleuTyrAsnSerAsnAsnLysLysTyrLeu
151016
Ala
    INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LEDSTH: 7 amino acids
iv Trib: umune aste

C. SIBANTEDNESS: sinche

T. Tobell (Fr. linear

12 if IBSTLE TYPE: postein
...i SYSTETICAL:
-iv) ANTI-SENSE:
(v) FFAGMENT TYPE:
-vi) OFIGINAL SOURCE:
(A) ORDANISM:
(P) STRAIN:
PO THE PUBLICATION OF BATE:
-R. HARLCITTE:
    TINGUE TYEE:
of CFLE TYPE: Hybridems producing human antibody GAH
(H. CELL LINE:
HIN OFGAMEDLE:
-vii: IMMEGIATE SCUECE:
A: LIPFAFY:
B - MeDE:
viii FOSITICH IN GENOME:
A THRAID FOME SEGMENT:
   IMA FORITION:
max) FEATURE:
AT DAME FEY:
\langle i_{1}\rangle \sim 1207747.7091
x) PUBLICATION INFORMATION:
A) ANTHORU:
\mathbb{R}^{2} - Piriæ:
 ') I UKNAL:
(D) 7DLUME:
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(E) ISSUE:
(F) PAGES:
(OH DATE:
(H DESCUMENT NUMBER:
(I - FILING DATE:
(U) FUBLICATION DATE:
THE PELEVANT FEBILIUES IN SEQ ID NO:
(EL) MEQUENCE DESCRIPTION: SEQ ID NO:20:
TrpAlastrTh:A: 4GluSer
#P- IMPORMATION FOR SEQ ID NO:21:
-i - SEQUENCE THAFACTERISTICS:
As IENUTH: 3 aming acids
 Pr TYPE: armny arid
   - SIBAMUEM NEGG: single
 10 I Hoth GY: Y.r.sr
 il) Math ME TYPE: protein
alic HYPETHETICAL:
 .7) AUTI-SENSE:
AND EFACULUT TYPE:
···L) - FIGHNAL COTRCE:
A DEPARTISM:
TE STEATM:
TO INTIMOMAL ISOLATE:
 DESERTED THE STAGE:
   HARLOTYFE:
    TIRSUF TYPE:
    THII TYPE: Hybridoms producing human antibody GAH
tH CELL LINE:
    GROWNELLE:
EVLIT HOMEDIATE SOURCE:
A DIREARY:
(B. CLONE:
coli: FORTION IN GENOME:
(A DEFORM SOME) SECMENT:
F MAR PERMISH:
of: THERE:
(ix) FEATURE:
(A) NAME/FEY:
OF ICCATION:
of Infunification Method:
   THEE INPOFMATION:
OR FURLIMATION:
(A A THORA:
AS THIRE
OF THEMAL:
(F) VOLUME:
IR - IBSUE:
(F) EAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(IT FILIME DATE:
(I FUBLICATION DATE:
OF RELEMBNIT FEBRUARS IN SEQ ID NO:
-ML) CEPTETOR DESCRIPTION: SEQ ID NO:21:
GinGlaly: 1y: SerTh: ProTrpThr
(); INFORMATION FOR SEQ ID NO:22:
+1 SEQUENCE CHAFACTERISTICS:
+A: LEMPTH: 10 amin.b acids
Fr TYPE: amuno acid
(7: SIEMANDEDNESS: single
(D) T FULLSY: 1.mear
(ii) HULECULE TYPE: protein
(iii) HYPOTHETICAL:
```

(iv) ANTI-SENSE:
-v) FRAGMENT TYPE:

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TLI PRIGINAL SOURCE:
·A OF BANISM:
(B) \in \mathbb{R}TkA\mathbb{H};
ed, indevioual isolate:
TEN HALLOTTEE:
** TIPS E TYPE:

** TIL TYPE: Hy: ridoma producing human antibody 1-3-1
(H: *ELL LINE:
(I' + F PATELLE:
(A) DIFFART:
TULL: POSITION IN GENOME:
VA- THE MILLSOME SEGMENT:
B IME E SITION:
·ix) FEATURE:
-A, DAME BEY:
(E) LowETION:
(C) IDENTIFICATION METHOD:
TO OTHER INFORMATION:
EMP FORDICATION INFORMATION:
A. ACTRONS:
   7.17.12E:
Ţ.
    NV BIKL:
   TRACTIFE:
   FA (E.):
(G) TATE:
(H) EGOUNEDT NUMBER:
(1) FILING LATE:
AT SUPLICATION DATE:
FOR PEDELANT PASTICLES IN SEQ ID NO:
(ML) SEQUENCE DESCRIPTION: SEQ ID NO:22:
IleS-rutiourStrly: IyeTrpGlyTrp

    THEOPMATION FOR SEQ ID NO:23:

   SEQUEDOT CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYFE: amino acid
+01 STRANLELNESS: single
I' TOPOLOGY: linear
 11) ModECTHE TYPE: protein
 This HYP THETICAL:
 in) ANTI-JENSE:
.Pr CTEAIN:
(n) INDIVIDUAL ISCHATE:
(E) HAPLETYPE:
FO TIJBVE TYPE:
 TELL TIPE: Hy: ridoma producing human antibody 1-3-1
(H) 'ELL LINE:
(I) DRGAMELLE:
(vii) IMMEDIATE SOURCE:
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(A) LIBRARY:
FF CLONE:
- viii) : FITI N IN GENOME:
(A) THE IN SOME (SEGMENT:
(B) MAL EUSITION:
(C) UNITS:
(IR) FEATURE:
(A) HAME/KEY:
(B) LOCATION:(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x) FUBLICATION INFORMATION:
(A) AUTHORS:
(B) MITTE:
(C) MOUNTAL:
(D) WOME:
AER ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NUMBER:
(I) FILITE DATE:
CON EMBLICATION DATE:
PRO SELEVANT RESTRUES IN SEQ ID NO:
(Mi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
11eGly3e::leTy:TyrSerGlySerThrTyrTyrAsnPro
1510
(I) INFORMATION FOR SEQ ID NO:24:
(i) JEQUEDOE CHARACTERISTICS:
(A) LFDGTH: 12 amino acids
(B) TYPE: amino acid
(C) STEAUDEDNESS: single
(D) TOPOLOGY: linear
(11) MOLECULE TYPE: protein
GART HYPOTHETICAL:
:171 ANTI-SENSE:
en FFAGREOT TYPE:
(vi) (ALIBIDAE, SOURCE:
GA: OFGADISM:
(E) STRAIN:
(C) INDIVIDUAL ISOLATE:
(E) DEVELOFMENTAL STAGE:
(E) HAFLOTYFE:
(F TISSUE TYPE: (C) (ELL TYPE: Hybridoms producing human antibody 1-3-1 (H (ELL LIME:
+1: 0F0MibblE:
(vii) IMMEDUATE SOURCE:
(A) ITERARY:
(E) CLONE:
(vili) FOSITION IN GENOME:
(A) CHROMOSOME SEGMENT:
(P) MAR BOSITION: OF TRUES:
(C. TUITS:
(ck) FEATURE:
(A - NAME FEY:
(F 16 ATION:
     ILENTIFICATION METHOD:
(I) OTHER INFORMATION:
(E) PUBLICATION INFORMATION:
(A) AUTHORS:
(F) TITLE:
 JEMAL:
 D: MOLUME:
E: ISSUE:
(F) PAGEG:
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(G DATE:
H DOCUMENT NUMBER:
(I FILING DATE:
(J & PLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:
wi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
G.ySe: TyrGlyGlyTyrTyrTyrGlyMetAspVal
1510

    INFORMATION FOR SEQ ID NO:25:

- E SECTENCE CHARACTERISTICS:
A JENSTH: 3 amin: acids
(B) TYPE: amino acid
  STŞANDBUNBUS: single
tf - I dellaβY: limear
11: MILETTAE TYPE: protein
-ili- HYESTHELLDAL:
(17) ANTI-CHIER:
AT PRABMENT IMPE:
(V1) (RIGINAL COURCE:
-A HEDANISM:
P CTRAIN:
  : INCINCONAT ISCIATE:
: CEVEL EMENTAD STAGE:
   HAELOTYPE:
   TISSUE TYPE:
cos CELL CYPE: Hybridoms producing human antibody 1-3-1 cH CELL CYPE:
+1) OFGANEGLE:
THE HEMBELATE SOURCE:
A DIBEARD:
P CHOOR:
wall FOSITION IN GENOME:
A: CHECHOSOME: SEGMENT:
HOME FORITION:
C UNITE:
 .x* FEATURE:
A NAME FEY:
G- DOMESTION:
(C | HENTIFICATION METHOD:
(D) OTHER INFORMATION:
(x PUBLICATION INFORMATION:
(A AUTHORS:
    TITLE:
| <u>L</u>.
   DOTRUAL:
1.0
   VOLUME:
   1. ....:
(F FAGED:
G TATE:
(H: DOCUMENT NUMBER:
(I) FILING DATE:
(C. FUBLICATION DATE:
(K) EFLEVANT FESIOUES IN SEQ ID NO:
(x): PEQUENCE DESCRIPTION: SEQ ID NO:25:
AppAlaLettEcclysGlnTyrAlaTyr
    INFORMATION FOR SEQ ID NO:26:
   SEQUENCE CHARACTERISTICS:
(A. LENGTH: 4 amino acids
(P) TYRE: aming adid
(0) STFANDEDNESS: single
(D) TOFOLOGY: linear
 ii) MULECULE TYPE: protein
(tii) HYPOTHETICAL:
( vi INTI-SENŠE:
(v) FRAGMENT TYPE:
```

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(vi) - RIGINAL SOURCE:
A) OF WANTSM:
BY FIRAIN:
D) OF FLIPMENTAL STAGE:
(E) HAR DUTYPE:
(F) TISCUE TYPE:
(3) CELL TYPE: Hybridoma producing human antibody 1-3-1
(H) CELL LINE:
(1) (FRAMELDE:
(vii) IMMEDIATE SOURCE:
(A) TIBERRY:
·B+ CIANDE:
"Li. FULLION IN GENOME:
AR THE MODIME, DEGMENT:
(B) MAR FIGUREOUS:
(2) 00000:
(1x) FEATURE:
(A) NAME REV:
(B) LOCATION:
FOR INECTIFICATION METHOD:
(1) ( ) HEE INFURNATION:
A) ACTHORU:
F) DITLE:
GC; GOURNAL:
(IO VOITME:
(E) ISSUE:
PPE PARPS:
car twik:
OHO GOTTUPUT NUMBER:
(A) FURLICATION DATE:
ON RECENTANT RESIDUES IN SEQ ID NO:
(Mi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
LysAsp. - r314
    INSUSTRATION FOR SEQ ID NO:27:
A: OFQUENCE CHARACTERISTICS:
(A LEDOTH: 11 amino acids
(E. TYPE: amino acid
00 STRANTEFNERW: single
00 TOHOLOW: linear
(it) IMIF "TE TYPE: protein
...i HYF.THETUCAL:
+17) AMT 1-MINCL:
FFARMEDT PYLE:
(vi) OFIGINAL COURCE:
(F) STRAIN:
*C INCLUDIAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
*E - HALLOTYPE:
· Fr TIDIUF TYEH:
Fig. TRIL IMPE: Hybridoms producing human antibody 1-3-1 He TRIL IMPE:
 I * * R ANEDLE:
 THE INTEDIATE SOUNCE:
AF DIHRARY:
For CLONE:
(viil) POSITION IN GENOME:
(A) CHROM (SOME/SEGMENT:
(B) MAP POSITION:
(C) UNITH:
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Tix, FEATURE:
(A NAME / KEY:
(B DECATION:
( : IDENTIFICATION METHOD:
for other information:
(x) FUBLICATION INFORMATION:
(A) AUTHORS:
E: TITLE:
   THIRDAL:
DOWN LITTLE :
   EAH.:
   1.842.
H IN COMENT NUMBER:
(I) FILING DATE:
AND FURILIDATION DATE:
(K) RELEVANT RESIDUED IN SEQ ID NO:
Mi) OFFICENCE DESCRIPTION: SEQ ID NO:27:
G.nS.:AlaAspSerSerGlyThrTyrGluVal
    ILP FMATIRN POR SEQ ID NO:28:
  THOUSE THARACTERISTICS:
LFNGTH: 24 base pairs
.F. TYFE: nucleic acid
40% CTRANCEDNESS: dauble
(E) Topology: linear
(11) MONETUR TYPE: GONA
IV) ADDI-SHUSE:
A OFWANISM:
F STEAIN:
OCE INDIVIDUAL ISOLATE:
(I DEVELOPMENTAL STAGE:
(F) HAFLOITPE:
(F TIRSUE TYPE:
00. CEIL TYPE: Hykridema producing human monoclonal
autil eg, an antigen to which exists on the surface of
dancer fell membrane
H OFFI TIME:
 1 (6 1238118):
wii: HIMEDHATE SOURCE:
A. DIESASY:
TEL COMER:
Tunit Presiden in GENOME:
(A) CHEDMOSOME BEGMENT:
PREMAR ESPETION:
-D: THIRD:
ix) FEATURE:
 A DAME KEY:
 B LOCATION:
    DEBLIFICATION METHOD:
    DIESE INFORMATION:
E PUBLICATION INFORMATION:
A ATTHES:
(B) TITLE:
(C) DURNAL:
(D) VOLUME:
(E) ISSUE:
(F) FAGES:
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(3) DATE:
HA BARDUMENT NUMBER:
· I · FILING DATE:
· J · F BLICATI N FATE:
-K FELENANT RESIDUES IN SEQ ID NO:
-M1) JEQ 'SNOT DESCRIPTION: SEQ ID NO:28:
ATCAS JASTWOTROTTWOTACTGG24
   INPURMATION FOR SEQ ID NO:29:
   SEQUENCE CHARACTERISTICS:
A' IEMSTH: 3e base pairs
-B TYLE: nucleic acid
401 STRANDEDNESS: double
Do Toffleway: linear
 .1) MINESTUR TYPE: CENA
 :Lik HYE THETICAL:
 EV: ANDI-JENUE:
   FEAR CENT TYPE:
tray - A DRIMAL FOORCE:
A) ORSAHIOM:
 B STEATH:
CO INCOMPANT ISOLATE:
DO DEVEN EMENTAL STAGE:
    HARLOTYFE:
 PO TIBBUR TYPE:
    CFLL TYFE: Hyrriaims producing numan monoclonal
antibody, an antipen to which exists on the surface of
cance: re.l membrane
H' CELL LINE:
 I DEGAMELLE:
 vai HIMPHATE SOURCE:
AN LIBRARY:
valide FOURTION IN GENOME:
 A THEFT SOME SERMENT:
 B MAF FORITION:
· (x) FEATURE:
A NAME FRY:
(C) IDENTIFICATION METHOD:
(D) OTHER INFURNATION:
OR - FURLICATION INFORMATION:
A ATTERNS:
   N 050741:
   7. . . . . . .
(F; ICSOR:
-F' FAGEC:
(B) CATE:
th: Desimbli Dumber:
I, FILING DATE:
(I) PUBLICATION DATE:
F REGEVANT RESIDUES IN SEQ ID NO:
Mi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
ATTIG FINE VATIOTATTA VA STEGNAGOACCTACTAC 3 6
    INF EMATION FOR SEQ ID NO:30:
    SELVEDOR CHARACTERISTICS:
As LEDVIE: 1. base pairs
the PYFE: mucleard adid
+7: STFANDEDNESS: doable
(D) TOPOLOGY: linear
(11) MULEPULE TYPE: cDNA
..ii) HYPOTHETECAL:
(tv) ANTI-SENJE:
(*) FRAGMENT TYPE:
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(vi) CPIGINAL SCURCE:
·A) (EGANISM:
(B) CTEATM:
the INCLUSIONAL ISCHATE:
DO LEWEL EMENTAL STAGE:
E. HALL IYPE:
:F: TICSUE TYPE:
.G. CELL TYPE: Hykridoma producing human monoclonal
antibody, an antigen to which exists on the surface of
cancer cell membrane
THA OFILE CIME:
 I - OFGANEILE:
.vii IMMEDIATE SOURCE:
A: LIPPARY:
B < 17 17 17 1
wii. HOUNTEN IN GENOME:
AT THESE NOME SEGMENT:
BE MAE POSITION:
de murs:
(ix) FEATURE:
JAH DAMES KEY:
   location:
notication method:
   OF THEF HUPGEMATION:
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A ATTEMPS:
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w1) 3FQUENCE IESCRIPTION: SEQ ID NO:30:
GEKRYRUMCEWOL
(1) INFOFMATION FOR SEQ ID NO:31:
(1) SEQUENCE CHARACTERISTICS:
(A) LFDOTH: 04 base pairs
(B) TYPE: nucleud acid
HAR STRANCENERS: double
   To be Legar Linear
AZOS BERTTE TYPE: SONA
(111) HYFOTHETICAL:
(17) AUTI-SENSE:
to FRACMENT LYPE:
twi) OFIGINAL COURCE:
(A) OF WALLSM:
B. STRAIN:
    INCULTUAL ISOLATE:
 D) DEWFLORMENTAL STAGE:
ED HARDSTYFE:
   TELL TYPE: Hybridoma producing human antibody GAH TELL LIME:
  R AND ME
 Wil HEEDLAFF SOURCE:
A: DIFFAFY: (B) DLOUE:
vii.) POSICION IN GENOME:
(A) CHEUMISUME SEGMENT:
(B) MAE POSITION:
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-wi) JEQUENCE DESCRIPTION: SEQ ID NO:31:
ATCAG PAGT FGTG FTTH GTAGTGG24
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(i) SEQUENCE CHARACTERISTICS:
   IFMOTH: 3: base pairs
    TYFF: :::cleir a mid
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T FOIGHT: Sucha:
 va) Mula CYLE TYPE: SONA
vai Hypotherican:
(iv) ANTI-SENSE:
IV FFACMENT TYPE:
.v.) * FIGINAL FOURCE:
A: OFGANISM:
 E STRAIN:
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 E HARD TYPE:
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GELL TYPE: Hykridoms producing human antibody GAH (H) CELL LINE:
(I - OPGAMELLE:
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OF CIRPE
Colif FOSITION IN GENOME:
FAR CHECIN COME, REGMENT:
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(IX) FEATURE:
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(I) FILING PATE:
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K) BELEVANT BESIDUES IN SEQ ID NO:
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+2* IMP*@MALION F & SEQ ID NO:33:
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.E TYPE: nucleic acid
C: STRANDEDNESS: double
 IC Tolob GY: linear
(11) MOLECULE TYPE: 60NA
.1117 HYPOTHETICAL:
 17) ANTI-SENSE:
 v FAARTENT TYPE:
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:00 DEVELORMENTAL STAGE:
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TOTACOORACTACGGGGGGGGGTGACTAC27
(c) INFORMATION FOR SEQ ID NO:34:
 . SEQUENCE CHARACTERISTICS:
 A THOUTH: 51 hase pairs
 F TYPE: nucleic acid
F STEAMMERMESS: double
Do I'lloh (Y: Lincar
(LL) MALETTEE TYPE: cDNA
.li) HYPITHETICAL:
(17) ANTI-CENSE:
(v) SFAGMENT TYPE:
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A: OF BANIEM:
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(C) INDIVIDUAL ISOLATE:
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(D) DEVELOPMENTAL STAGE:

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(F HAPLOIYPE:
JE TIBSTE TYPE:
(G. CELL TYPE: Hybridoma producing human antibody GAH
HE CELL LINE:
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("iii" EGGITION IN GENOME:
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ME) SEQUENCE DESCRIPTION: SEQ ID NO:34: AAGTOTAG TA SAGTGTITTATACAACTCC30 AACAATAGAACTCC30:
   THE EMATION FOR SEQ ID NO:35:
    SEQUEDOR CHARACTERISTICS:
    IENGIE: 21 base pairs
(B) TYPE: Madletc area
OF STEAMERANE double
     Defender: Linear
FIL) DOLE THE THEF: SONA
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·iv) ADTI-CFNSE:
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*1 INFORMATION FOR SEQ ID NO:36:
(i) SEQUENCE CHARACTERISTICS:
(A IFDOTH: 2" have paigs
dr. THE: molece acid
   STRANCFONEDS: oxuble
of the Tolke Double Clinear
 (11) MODET THE TYPE: BONA
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G CFIL TYPE: Hyrricoms producing human antibody GAH
(vii) IMMEDIATE SOURCE:
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(MI) JEQUENCE DESCRIPTION: SEQ ID NO:36:
CAGCAGTATTATAGTACTCCGTGGACG27
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(.) INFORMATION FOR SEQ ID NO:37:
(1) SEQUENCE CHARACTERISTICS:
AA LEEDHE: 30 hase pairs
B TYPE: nucle.c acid
er structurents: acable
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 11) MODETULE THEE: COMA
 idi: HYPOTHETICAL:
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(M.) TENTETICE DESCRIPTION: SEQ ID NO:37: ATCAST WOTA FTANTITATING (GGGCTGG3)
i. ILBÖHMATION FOR SEQ ID NO:38:
c. SPONEDOF CHARAGIERISTICS:
(A) LENGTH: 40 hase pairs
(B) TYFE: nucleic asid
(C) STRANDEDNESS: double
(I) TOPOLOGY: linear
(11) IN LETTLE TYPE: SONA
(TLI) HYLOTHEFICAL:
-17) AUTI-SFNSE:
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-wi) (41 TIMAL SOURCE:
·A· CETAILSN:
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(C) INDIVIDUAL ISOLATE:
.10 DEVELOPMENTAL STAGE:
E' HAPIDTYFE:
   TIJSUE TYPE:
(3) CHIL TYPE: Hybridoma producing human antibody 1+3-1
(H) CELL LINE:
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(I) ORGANELLE:
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I: IMPORMATION FOR DEQ ID NO:39:
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A) IEDOTH: 36 base pairs
Ex TYPE: nucleic acid
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L: TOROLOGY: linear
(11) MOUECULE TYPE: GDNA
Fili HYPOTHETICAL:
.va) ADDI = CEDISE:
A PRIMITINE
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(D) DEVELOPMENTAL STAGE:
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4 HIL TYPE: Hyrridoma producing human antibody 1-3-1
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(xi) JEQUENCE DESCRIPTION: SEQ ID NO:39:
GGGAUUTADGGGGGTACTACTACGGTATGGACGTC36
(A) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LEMETH: 27 base pairs
(B) IMPE: nucleic acid
 (1) THEMPAY: linear
(.i) MOLECULE TYPE: dINA
esii HYSCTHETICAL:
erve ADDI-JENSE:
.v. BBASKEUT LYPE:
(v1) FRIGHMAL FOURCE:
(A) GEGANISM:
(A) OF SEAL CONTROL OF STAGE:
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(3) OF VERY PHENDAL STAGE:
    HARLUTTER:
 F TISSUE TYPE: Hybridema producing human antibody 1-3-1
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(A) DIBBARY:
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E) BELFVANT RESIDUES IN SEQ ID NO:
ME) CREMENTE DESCRIPTION: SEQ ID NO:40:
GWTG MAIL GWAAAGDAATATGOTTAT27
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 i) DEQUEEDER CHARACTERISTICS:
(A) LEÑ VTH: 12 pase pairs
 B) TYFE: nucleic abid
 (C) STRANDEDNESS: double
 (D) ToPoLoG7: linear
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(ii) MoLECULE TYPE: SDNA
(iii) HYPOTHETICAL:
·iv) AUTI-JEMSE:
(v) FER MEIT TYPE:
. . i. CRIGINAL BOURCE:
(A) OF BANIUM:
(B) CTFAIN:
WAR INDIVIDUAL ISOLATE:
(F) CEVELOFMENTAL STAGE:
(F) HAPLOTYPE:
(F) HISSUE TYPE:
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AAAGAMA STGAG1
(I) INFORMATION FOR SEQ ID NO:42:
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(A) LEMBTH: 33 base pairs
 E THE: mid. Aid acid
 C DIFAMIFUMEDS: double
 De 10:000000: .inear
:1) DOUGHTHE TYPE: coNA
Fili HYP THELICAL:
(17) AUTI-SEMSE:
(v) FRACHENT TYPE:
(VI) OFIGINAL SOURCE:
(A) OFGANISM:
(E) CTRAID:
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 D. DEVELORMENTAL STAGE:
 E HARLOTYPE:
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    TELL TYPE: Hyperidoma producing human antibody 1-3-1
 H GHILL LINE:
*11 ORBANELLE:
 vii) IMMEDIATE SOURCE:
 A) DIFFRARY:
 B) CLINE:
(viii) FOSITIUM IN GENOME:
(A) CHROMUSOME/SEGMENT:
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 File: USPT

Nov 23, 1993

US-PAT-NQ: 5264221

DOCUMENT-IDENTIFIER: US 5264221 A

TITLE: Drug-containing protein-bonded liposome

DATE-ISSUED: November 23, 1993

INVENTOR-INFORMATION:

NAME CITY STATE ZIP CODE COUNTRY

Tagawa; ToshiakiYokohamaJPHosokawa; SaikoKawasakiJPNagaike; KazuhiroSagamiharaJP

ASSIGNEE-INFORMATION:

NAME CITY STATE ZIP CODE COUNTRY TYPE CODE

Mitsubishi Kasei Corporation Tokyo JP 03

APPL-NO: 07/ 886846 [PALM]
DATE FILED: May 22, 1992

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY APPL-NO APPL-DATE

JP 3-118762 May 23, 1991

INT-CL: [05] A61K 9/127

US-CL-ISSUED: 424/450; 428/402.2, 436/829, 935/54, 530/812

US-CL-CURRENT: 424/450; 428/402.2, 436/829, 530/812

FIELD-OF-SEARCH: 424/450, 424/417, 436/829, 428/402.2, 264/4.1, 530/402, 530/403,

530/810, 530/812, 935/54

PPIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search ALL

PAT-NO ISSUE-DATE PATENTEE-NAME US-CL 4429008 January 1984 Martin 424/450

§ 5059421 October 1991 Loughrey et al. 424/418

Search Selected

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO PUBN-DATE COUNTRY US-CL

0354855 February 1990 EP 9004384 May 1990 WO

OTHER PUBLICATIONS

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Klibanov. FEBS 268, p. 235 1990.

Biochimica et Biophysica Acta, 1062, pp. 142-148, 1991, A. L. Klibanov, et al., "Activity of Amphipathic Poly(ethylene glycol) 5000 to Prolong the Circulation Time of Liposomes Depends on the Liposome Size and is Unfavorable for Immunoliposome Binding to Target".

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Vesicles".
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Tumor-Specific Monoclonal Immunoblobulin M Antibody.sub.1 ". Cancer Research, vol. 47, pp. 4471-4477, Aug. 15, 1987, H. Konno, et al., "Antitumor

Effect of Adriamycin Entrapped in Liposomes Conjugated with Anti-Human Alpha-Fetoprotein Monoclonal Antibody.sub.1 ".

FEBS Letters, vol. 268, No. 1, pp. 235-237, Jul., 1990, A. L. Klibanov, et al., "Amphipathic Polyethyleneglycols Effectively Prolong the Circulation Time of Liposomes".

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World Patent Index Latest, No. 89-335876.

ART-UNIT: 152

PRIMARY-EXAMINER: Page; Thurman K.

ASSISTANT-EXAMINER: Kishore; G. S.

ABSTRACT:

A drug-containing protein-bonded liposome comprising a liposome containing a drug and having maleimide residues on its surface, and a protein and residues of a compound having a polyalkylene glycol moiety, bonded via respective thiol groups to the maleimide residues.

15 Claims, 2 Drawing figures Exemplary Claim Number: 1 Number of Drawing Sheets: 2

BRIEF SUMMARY:

- The present invention relates to selective chemotherapeutic drugs for various diseases including cancer. More particularly, it relates to a drug-containing protein-bonded liposome.
- A missile therapeutic agent whereby a drug can be concentrated at a required active site utilizing a specific reactivity of an antibody, is expected to be useful in various medical fields including a field of cancer treatment in view of its high level of effectiveness and low side effects. For realization of such a missile therapeutic agent, it is important to establish a technology for

combining an antibody and a drug. Heretofore, it has been attempted to bond an antibody and a drug by a method wherein a drug is cherically modified for bonding i.e. a method wherein an antibody and a drug are directly bonded, or a method wherein they are bonded via a water-soluble polymer such as dextran. However, with these methods, problems have been pointed out that the amount of a drug which can be bended per molecule of an antibody is small, and the activities tend to be reduced by the modification of a drug. On the other hand, as a means for transporting a drug in a large amount without modifying the drug, a method has been proposed wherein a drug is contained in a liposome and an antibody is binded to the surface of the liposome. Namely, an antibody-bonded liposome has been proposed.

- Also in the field of cancer treatment, anti-cancer drug-containing antihody-bended liposomes have been prepared, and many research institutes have reported excellent antitumor effects thereof (Konno et al., Cancer Res. 47, 44°1 (1:87), Hashimoto et al., Japanese Unexamined Patent Publication No. 1500:27(98)). However, at the same time, sine problems of antibody-bonded liposomes have been pointed out. Namely, many if antihody-bonded liposomes administered are likely to be captured by organs of retibulo-ordenelial system such as liver and spleen, whereby he dacquate offects tend to be obtained (Hashimoto et al., Dancer Res. 43°63(8), 1889)).
- On the other nand, it has been proposed to bonse.g. polyethylene glydol to a liptsime as a methic of improving the general properties of a liptsome, such as leakage of the contained substance, agglomeration and a nature of being captured by organs of retiralcendothelial system (Japanese Unexamined Patent Publications No. 249717/1989 and No. 149813 1981, Alexander L. Klibanov et al. FERS letters 268 235 (1990)).
- However, in these methods, lipophilis derivatives of polyethylene glycol and a compound such as a long chain alighatic acid are mixed with other liposome-constituting lipids, and a polyethylene glycol layer is formed on the liposome surface during the preparation of the liposome, or polyethylene glycol derivatives reactive with amino groups are attached to amino groups introduced to the liposome surface. When such methods are applied to an antibody bonded liposome, bonding of the antibody is likely to be hindered by the polyethylene glycol layer already formed on its surface, or deactivation of the antibody is likely to result. Therefore, the conventional method of incorporating polyethylene glycol has not been primarily intended for application to an antibody-bonded liposome.
- The present inventors have conducted extensive studies to present a drug-containing antibody-bonded lipusome having the nature of being daptured in the retoral bendithedial system improved, and as a result, have found it possible to appumption this object by firstly reacting a process to which a third group is imparted (a third modified postwin) to a lipusome having malermine proups and then reacting a compound having a mojety of a polyalkylene glycel to which a third group is imported (a third-modified polyalkylene glycel) to the remaining malermine groups.
- Thus, the present invention provides a drug-containing protein-bonded liposome comprising a liposome containing a drug and naming maleimide residues on its surface, and a protein and residues of a compound having a polyalkylene glycol modely, bonded via respective thiol groups to the maleimide residues.

DRAWING DESCRIPTION:

In the administrating drawings:

FIG. 1 shows the reactivities of antibody-bonded liposomes to numan gastric cancer cell line MKN 45, with respect to polyethylene glycol (PEG)-modified and non-modified liposomes.

The ordinate represents the amount bonded to MKN 45 in terms of the amount of 6-carboxyfluorescein (CF). In this Figure, lip represents the CF-loaded liposome, and antibody-bonded lip represents the CF-loaded antibody-bonded liposome.

FIG. 2 shows the antitumor activities of the adriamycin-containing antibody-bonded PEG-modified liposome against cancer transplanted to nude mouse. The abscirsa represents the number of days after initiation of the therapoutic test, and the ordinate represents the assumed tumor weight. In this Figure, represents the day on which the drug was administered; FBS represents a phosphate buffer physiological saline; ADE represents adriamycin alone; FM represents the adriamycin-containing PEG-modified liposome, and FM(GAH) represents the adriamycin-containing human monoclonal antibody-bonded PEG-modified liposome.

DETAILED DESCRIPTION:

- A New, the present invention will be described in detail with reference to the preferred embediments.
- 2 (1) Lipesome
- 3 I The liposome is composed essentially of phosphatidyl choline, cholesterol and matermide-modified phosphatidyl ethanolamine. However, a phosphatidic acid such as dipalmitoylphosphatidic acid (DPPA) or the like may be incorporated as a substance imparting an electric charge.
- As a preferred lipisime, a liposome composed of dipalmitoylphosphatidyl pholine (DPEC), pholesterol (Chol) and maleimide-modified dipalmitoylphosphatidyl ethanolamine (maleimide-modified DPPE), may be mentioned.
- 5 If The maleimide-modified phosphatidyl ethanolamine can be obtained by the reaction of a maleimide-containing compound reactive with an amino group, with an amino group of prosphatidyl ethanolamine (FE). The maleimide-containing compound may be N-(.epsilon.-maleimidocaproyloxy)succinimide, N-succinimidyl 4-(p-maleimidophenyl)putyrate, N-succinimidyl 4-(p-maleimidophenyl)propionate or N-(.gamma.-maleimidobutylyloxy)succinimide. FE may be dipalmitoylphosphatidyl ethanolamine.
- 6 If The respective components are used in such proportions that per mode of the phosphaticyl choline, cholesterol is used in an amount of from 0.3 to 1 mol, preferably from 0.4 to 0.6 mol, the maleimide-modified phosphaticyl ethanolamine is used in an amount of from 0.31 to 0.2 mol, preferably from 0.02 to 1.1 mol, and the phosphaticid acid is used in an amount of from 0 to 0.4 mol, preferably from 0 to 0.15 mol.
- 4 For the preparation of the liposoms, a conventional method can be used. For example, a lipto mixture having a solvent removed, is hydrated and emplished by a homogenizor, followed by freezing-thawing to obtain a multilamelia liposome. To further adjust it to a proper particle size, it may be subjected to supersonic treatment, high speed homogenizing or press-filtration by a membrane having uniform pores (Hope M. J. et al., Biconimica et Biophysica Acta :13, 85 (1985)).
- A preferred size of the liposome is not larger than 300 nm, more preferably from 50 to 100 nm.
- 9 (2 Drud
- 10 I As the drug, an antitumor drug such as adriamyoin, dampomyoin, mitomyoin, displatin, minoristine, epirubicin, methotremate, 5FU or adlacinomyoin, an aminoglucoside such as gentamicin, a .beta.-lactam antibiotic such as sulpenisillin, a toxin such as ricin A or diphtheria toxin, antisense ENA

- against HIV or ras gene, or actinoplane (Polycytlic xanthones produced from actinoplane E-314 (E. Kobayashi et al., J. Antibictics 41, 741 (1988)), may be employed.
- If Loading of the drug into the liposome can be conducted by hydrating the lipid with an aqueous drug solution in the base of a water-soluble drug, or by mixing the drug and the lapid in a volatile organic solvent, followed by distilling the solvent off and hydrating the mixture of the drug and the lipid to embed the drug in the liposome, in the base of a fat-soluble drug. Further, in the base of addiamyoun, daunomyour or epirubicin, leading can be conducted by a remote loading method utilizing a pH gradient (Lawrence D. Mayer et al., Cancer Res. 49, Edd. (1989)).
- 12 (3) Thiol-Modified Pr. tein
- 13 I As the protein bonded to the lip some, various physiologically active substances soluting an antibody, PGF and EGF, may be employed. Preferred is an antibody. The antibody is an antibody reactive with the virus, bacteria, calls or tissue to be treated. For example, polyclonal antibodies of various animals, a notice mondelenal antibody, a human-mouse chimeric intibody and a human monoclonal antibody may be employed. Among them, a human monoclonal antibody is preferred in the sense that it is not a protein of a foreign animal.
- Introduct: n of third groups to the protein can be conducted by a method wherein a compound is employed which is commonly used for third-modification of a protein and which is reactive with an amino group of the protein, such as N-subclimitary-3-(1-pyriay) dith. Coropionate (SCOE) (Carlsson, J. et al., biochem. J. 174, 713-1874) or immothiclane, marcaptialkylimidate (Traut, R. E. et al., biochemistory ls., 5206-1877)), or the like. In the case of an antibody, a pethod may be employed wherein endogenous althird groups are reciped to third groups. For bonding an antibody and a liposome, the latter method utilizing endogenous third groups is preferred from the viewpoint of the maintenance of the activities. When IgG is employed, it is subjected to Piah's sub.3 modification with an enzyme such as pepsin, followed by reduction with e.g. dishipting its to obtain Fab', whereupon third groups formed in Pab' are subjected to the finding reaction with the liposome (Martin, F. J. et al., biochemistory, 25, 4219 (1981)). In the case of IgM, J-chain is reduced under a mild condition in accordance with a method of Miller et al. (1. Biol. Them. ii., 256 (1885), whereupon third groups of Folioty of IgMs thereby obtained, are subjected to the rending reaction with the liposome.
- 3 Bending of the malermide group-bontaining lippsome and the thisl-modified protein can be arounglished by reacting them in a neutral buffer solution pH(0,0,0) to 7.5 for from 1 to 10 nours.
- 16 4) Ommound Containing a Thiol-Modified Polyalkylene Blycol (Moiety
- 17 I As the polyalkylene glycol molety of the compound, polyethylene glycol or polypropylene glycol may, for example, be mentioned. Preferred is polyethylene glycol, and its meacee of polymerication is preferably from 21 to 400.
- 18 of Polintroduce third groups to polyalkylene glyppls, various third-modification reactions which are commonly used for hydroxyl groups, amino groups, carboxyl groups and triazine, may be employed. Specific examples will be given below with respect to the case of polyethylene glyppl, but it should be understood that the precent invention is by no means restricted by such specific examples.
- 19 Namely, there are a method wherein monometroxypolyoxy ethyleneamine and various thioldarpoxylic acids are dehydrated and condensed, a method wherein pyridyl dithioproplomyl broup is introduced into monometroxypolyoxy ethyleneamine by SPDP, followed by reduction, a method wherein thiol is introduced into monomethoxypolyoxy ethyleneamine by immothiorane, a method wherein various thiolamines are bonded to active esters of monomethoxypolyoxy

- ethylenecarboxylic acid, and a method wherein a polyethylene glycol triazine derivative is bonded to thiolamine.
- 20 More specifically, as shown in the following Example, 2,4-bis(polyethylene 4.you)-d-ohlord-s-triazine (activated PEGII, manofactured by S-ikagaku Kogyo K.K., is reacted with systeine, followed by reduction to obtain a systeine-bonded activated PEGII.
- 21 Cupporting the Thiol-Modified Protein and the Compound Containing the Thiol-Modified Polyalkylene Glycol Modety on the Surface of the Loposome
- To bond the thick-modified protein and the compound containing the thick-modified polyalkylene glycol modety to the surface of the Liposome, firstly, the thick-modified protein is added and reacted in a neutral buffer solution to the liposome having an excess amount of maleimide groups. For example, in the case of a thick-modified antibody, the thick-modified antibody is employed in an amount of firm 0.1% mol to 10% mol per mol of maleimide modes. Then, to the remaining maleimide groups, an excess amount of the thick-modified polyalkylene glycol, preferably in an amount of at least twice in equivalent, is added to obtain an antibody-bonded polyalkylene glycol-modified liposome. By this process, it is possible to accomplish the blocking effects of excess remaining maleimide groups.
- 23 of Method of Use of the Drug-Containing Protein-Bonded Liposome
- The grup-pointaining protein-bonded lipesome thus obtained, such as an againmyoun-containing antibody-bonded PED-modified lipesome, may be formulated into a doubley a conventional method such as a denyuration method (Japanese PCT Publication No. 502:4* 1990), a method wherein a stabilizer is added to form a liquid formulation (Japanese Thexamined Patent Publication No. 43:1/1/39) or a freeze-drying method (Japanese Unexamined Patent Publication No. 3931 1989).
- The drug thus formulated can be used by e.g. intravascular administration or local administration such as intravesical or intraperitoneal administration administration administration amount may be optimally selected depending on the drug contained in liposome.
- In the rase of an admixing tintaining liptsome, as an example, the dose is usually at most 50 mg/kg, presentably at most 10 mg/kg, more presentably at most 5 mg/kg, as the amount of admixing.
- 27 If w, the present invention will be described in further detail with reference to Enamples. However, it should be understood that the present invention is by a means restricted to such specific Examples.
- 28 EMAM! 1.E. 1
- 29 Cinfirmation of the Effect Whereby PEG-Modified Adriamydin-Containing Lipesome Avoids he Reticulpendothelial System
- 30 Preparation of Thick-Modified Polyethylene Glybbl
- 4- mg of 1-dysteine was dissolved in a 0.4M borio acid buffer solution. Then, 200 mg of 1.4-bis(polyethyleneglycol)-6-chloro-s-triazine (activated PEGII, manufactured by Seikapaku Rogyo K.K.) was added thereto, and the mixture was reacted at room temperature overnight. To the dysteine-bonded EFG thus obtained, all mg of dishlothrestol (DTI) was added, and the mixture was reacted at 30 degree. Of for 4 hours to obtain a solution containing a systeine-bonded PEG. The reaction solution was further desalted by gel filtration on GE-21 column (manufactured by Seikagaku Rogyo E.K.), and the solvent was replaced by 1 mM prosphate buffer solution pE7.4 and 0.15M NaCl (PBS). Then, the solution was added to 7 ml of thiopropylsephanose 6B (Pharmacia) equilibrated with PBS. Non-bonded substance was removed by washing with PBS. The dysteine-bonded PEG

- bonded to the gel was eluted with PBS containing 50 mM DTT. Then, excess DTT was removed by gel filtration to obtain the above-identified product.
- 32 Preparation of Maleimide-Modified Dipalmit: ylphosphatidyl Ethanolamine
- 117 mg of dipalmitoylphosphatidyl ethanolamine, 80 mg of G-(.epsilon.-maleimidocarproylomy) subdinimide (EMCS) and 44 ul of triethylamine were added to a chloroform solution containing 1/1 of methanol and reacted under a nitrogen stream. Three hours later, 20 mg of EMCS was further added, and the mixture was further reacted at rion temperature for 3 hours.
- After confirming that the minhydrin reaction of the reaction solution became negative, the reaction solution was evaporated to dryness under reduced pressure, and the product was dissolved again in a small amount of onlinoform.
- 35 The maleimide-modified dipalmitoylphosphatidyl ethanolamine was purified by chromatography using UNISIL (manufactured by Gaschro Kodyo E.E.). Damely, the product was added to the column equilibrated with oblight form and developed with an eluting solution of oblightform/methanol-10/1 to obtain the desired substance.
- 36 Preparation of Maleimode-Containing Adriamydin-Loaded Diposome
- 37 I ml of a 0.5M citric acid buffer solution pH4 was added to 100 mg of a solid .ipid mixture of DEPO/chol.OPPA.maleimids-modified DPPE/010/10/2/0.1 (mol ratio) manufactured by Nippon Seika E.E.), and the mixture was stirred. Then, treezing-thawing was repeated five times for hydration to optain a multi-lame.la liposome. Then, the multi-lamella liposome was subjected to press-filtration ten times while heating at 60 degree. C. by a press expanatus sextruder, manufactured by Liber Biomembranes) provided with a polyparhonate membrane having a pore size of 200 nm (nucleopore, Microscience), to obtain a liposome having a regulated particle size. This liposome solution was neutralized with a 1M NaOH solution. While heating the imposome solution at (0) degree. C., adriamybin (manufabtured by Kyowa Hakko) was added in an amount of 1/10 by weight of the lipid. At least 97 of admiamyoin was actively loaded to the lippsome in accordance with the pH gradient between the interior and exterior of the liposome, to obtain a maleimide-containing admissiydin loaded liposome.
- 38 Introduction of Thiol-Modified PEG to the Maleimide-Containing Liposoms
- 39 To the above maleimode-containing liposome, 5 .mu.mol of thiol-modified PEG was added, and the mixture was reacted in PBS at room temperature for 6 hours to obtain a FEG-modified admissiprin-loaded liposome. Further, the liposome was subjected to jet filtration by sephanose CL(B) pharmacia) to separate unreacted cysteine-bonded PEG, followed by evaluation test.
- 40 Study of Intracorporeal Behavior
- 41 The prepared liposome was intravenously administered to a mouse from the tail in an amount of 5 mg/kg as adminisped, and 30 minutes later, the mouse was killed, and adminisped in each of the extracted organs was extracted and quantitatively analyzed in accordance with the method of Konno et al.
- Mamely, each organ was homogenized in a 0.3M hydrochloric acid, 50 ethanol, heated and centrifugally separated, whereupon the supernatant was measured by fluorescence of Ex 430 hm and Em 530 hm.
- 43 As shown in Table 1, a decrease in the amount of adriamycin in the liver and spleen was observed, and maintenance of a high concentration in the blood was observed.

TAPLE 1

Concentration of adriamycin in the respective organs upon expiration of 30 minutes from the administration (ug/g tissue) free ADR lip = ADR PEG-lip = ADR.

Blood	0.2	9.7	17.8
Liver	19.2	31.8	17.3
Spileen	7.2	110.1	90.0
Ling	7.3	4.2	5.5
Heart	3.2	1.5	3.5
Red	1.4	0.3	0.5
Brain	v ²	0.1	0.3

- 44 EXAMPLE 2
- 45 Confirmation of the Reactivity of the Antibody-Bonded PEG-Modified Liposome
- 1 ml of a 0.1M 6-carboxyfluorescein as a fluorescent marker was added to 100 mg of a solid lipid mixture (manufactured by Mippon Seika) of DPPC/cool/maleimide-modified DPPE=18/10/0.5 (mol ratio), and the mixture was hydrated and the particle size adjusted in the same manner as in Example 1 to optain a maleimide-containing fluorescent gye-loaded liposome.
- 47 Preparation of a Thiel-Modified Antibody
- 48 To an antitumor mouse monoplonal antibody (190), 1/40 mol amount of pepsin (Cooper Biomedical) in 0.1M acetic acid buffer solution pH 3.5, was added, and the mixture was reacted at 37.degree. C. overnight for digestion to obtain F(ab').sub.2. Further, by chromatography separation with a cationic exchange restrictment S, manufactured by Pharmatia., F(ab' .sub.2 was isolated. The separation was conducted by a linear gradient of from OM to 1.3M NaCl in a 0.1M acetic acid buffer solution pH4.0.
- To reduce it to Fab', 11 di of 11% DTT was added per mg of the antibody in a 0.1M acetic acid buffer solution containing 0.18M NaOl (pH 4.8), and the mixture was left to stand at room temperature for 80 minutes. After completion of the reaction, demineralization was conducted by gel filtration on FD-10 column (manufactured by Fharmacia) equilibrated with PBS to obtain Fab'. To the lipostra obtained from 110 mg of the above lipid, 5 mg of Fh' was added, and the mixture was reacted at 37.degree. C. for 8 hours and further 6 until of thick-modified polyathy, ene glycel was added to react it with excess maleimide, to obtain an antibody-binded FEG-modified liposome.
- 50 Confirmation of the Bonding Activity of the BEG-Modified Antibody-Bonded Liposome
- 51 Using numan gastoric cancer cell line MEC 45, of which the reactivity of the used mencolonal antibody had been confirmed, the reactivity of the PEG-modified antibody bonded liposome was confirmed in vitro.
- The above carboxyfluorespein-loaped antibody-bonded PEG-modified liposome was added to fitimes.10.sup.: cells of MKN 41 floated in trypcin, and the mixture was reacted in 90% numan inactivated serum at 37.degree. C. for 230 minutes. The centrifugal pellet of cells was washed with PBS, and then carboxyfluorescein was freed at 60.degree. C. with 10% triton.times.100, and the amount bonded to the cells was calculated by a fluorescence measurement.
- 53 As shown in FIG. 1, a high reactivity with the opjective cells was observed

also in the case of the antibody-bonded PEG-modified 1:posome.

- 54 EKAMPLE 3
- 55 Confirmation of the Fharmacol great Activities of the Adriamycin-Loaded Monoclonal Antibody-Bonded EEG-Modified Licesome
- A solid lipid mixture of DPSC (mol/maleimide-modified DPPE-18/10/0.5 (mol patio) was treated in the same manner as in Example 1 to obtain an adriamydin-loaded maleimide-containing liposome.
- 57 Using a numer monoclinal antibody (IgG), Fab'-modified antibody was obtained in the same manner as in Example 1 except that the pH for the popsis digestion was changed to 4.0, and it was subjected to the bonding with the liposome. Further, it was modified by thip-modified PEG in the same manner to obtain an agrically-loaded numer monoclonal antibody-bonded PEG-modified liposome.
- 59 Using human cancer cell line MEM 45 of which the reactivity with the antibody was observed in vitro, and admiralation was observed in vivo with respect to the nude mouse-transplanted system, the antitumor applications were studied.
- For a therapelitic test, 1.times.10.sup.6 calls of MRN 45 cultured, were subcutaneously transplanted to a nude noise, and the therapelitic test was initiated when the weight of the tumor became about 101 mg ten days later. On the first day, the fourth day and the ninth day from the initiation of the therapy, the liposome was intravenously administered to the nouse from the tail in an amount of 5 mg/kg as administered to the change with time of the proliferation of the tumor, an assumed tumor weight was obtained by a calculation formula of short downeter.times.short diameter.times.long diameter/2 of the tumor in accordance with a Battelle Columbus method, and the change with time was shown using as a reference the weight of the tumor at the initiation of the therapy.
- 61 As a result, as shown in FIG. ., strong antitumor activities of the adriamydin-loaded monoplonal antibody-bonded PEG-modified liposome were shown.
- With the liposome obtained by the present invention, it is possible to suppress the non-specific dapture in the reticuloendothelial system such as liver or spleen as observed with the otherntional liposomes, and thus the liposome of the present invention is effective for use as a selective chamotherapeutic drug, particularly as a cancer treating using.

CLAIMS:

We claim:

- 1. A drug containing, protein-bonded liposome, which comprises a liposome containing a drug, said liposome having maleimide groups on the surface thereof, wherein a portion of the male.mide groups are bonded to a thiol group-containing protein and a remaining portion of the male:mide groups are conded to a thiol group-containing polyethylene glycol moiety; said liposome comprising prosphatidyl sholing, sholesterol and maleimide-modified chosonatility ethanolamine.
- 2. The drig-containing, protein-bonded liposome of claim 1, wherein the protein is selected from the group consisting of an antibody, FGF and EGF.
- 3. The drug-containing, protein-bonded liposome of claim 2, wherein said protein is an antibody selected from the group consisting of animal polyclonal antibodies, mouse monoclonal antibodies, human-mouse chimeric antibodies and

human monoclonal antibodies.

- 4. The drug-containing, protein-bonded 1.posome of claim 3, wherein said protein is a numan monoclonal antibody.
- 5. The drug-containing, protein-binded liposome of claim 1, wherein the iposome comprises dipalmit cylphosphatidyl choline, cholesterol and haleimide-modified dipalmit cylphosphatidyl ethanolamine.
- 6. The drug-containing, pritein-bonded liposome of claim 5, wherein the maleimide-modified dipalmitoylphosphatidyl ethanolamine is obtained by reacting N-(.epsilon,-maleimidopapriyloxy) subcinimide and dipalmitoylphosphatidyl ethanolamine.
- The drug-containing, protein-bonded hoposome of claim 1, wherein the thiol group-containing protein haming maleimide groups bonded thereto is obtained by reacting maleimide residues on the liposome surface and a thiol moup-containing protein.
- t. The drig-containing, pritwin-binded liposome of claim 1, wherein said thill-group containing polyethylene glycol moiety having malehmide groups bonded thereto is obtained by reacting malehmide residues on the liposome surface and a thiol-group containing polyethylene glycol.
- . The drug-containing, protein-binded liposome of claim 1, wherein said drug comprises an antitumor drug, a .beta.-lactam antibiotic, a toxin, an aminoplaceside, antisense ENA or actinoplane.
- 10. The drug-containing, protein-condet siposome of claim 0, wherein the antitumor drug is selected from the group consisting of adriamydin, daunbmydin, nitomydin, displatin, vinoristine, epirubidin, methodrexate, 8-FU and adladinomydin.
- 11. The drug containing, protein-bonded Liposome of claim 9, wherein said aminogluposide is gentamicin.
- 12. The drug containing, protein-bonded liposome of claim 9, wherein said opera.-lastam antibiotic is sulpenisillin.
- 13. The drug containing, protein-bonded Liposome of claim 9, wherein said toxin is right. A or diphtheria toxin.
- 14. The drug containing, protein-bonded liposome of claim 9, wherein said antisense ENA is antisense ENA against HIV or ras gene.
- li. An antitumor drug, compressing a liposome containing a drug, said liposome having maleimide groups on the surface thereof, wherein a portion of the raleimide groups are bonded to a third group-containing protein and a remaining portion of the maleimide groups are binded to a third group-containing polyethylene glucol moiety; said liposome comprising phosphatidyl choline, cholesterol and maleimide-modified phosphatidyl ehtanolamine.